UNIVERSITY OF ALBERTA

On a Molecular Mechanism of Adaptive Mutation in *Escherichia coli*

by

REUBEN STEWART HARRIS ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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"Dich im unendlichen zu finden
Mußt unterscheiden und dann verbinden"

Before the infinite can be thine
One must first break it down and then re-combine*[*]

Goethe

---

[*] Source of quotation and translation: W. Johannsen in an article entitled "Heredity in populations and pure lines", p.26 in Peters, J. A. (1959) *Classic Papers in Genetics*. Prentice-Hall Inc., Englewood Cliffs, New Jersey

ABSTRACT

Adaptive mutations occur in non-growing or slowly growing cells in response to a non-lethal genetic selection, and were detected only in genes whose functions were selected. Adaptive mutations have been a subject of interest and debate for two major reasons: (i) the possibility that mutations could be directed to selected genes challenges the well-established doctrine that mutations are random; and (ii) their occurrence in apparently non-dividing cells contradicts the presumed association of mutation with cell generations. This thesis was undertaken with the goal of elucidating a molecular mechanism of adaptive mutation, a requisite for distinguishing adaptive mutation from growth-dependent mutation.

Studies on the molecular mechanism of adaptive reversion of an episomal *lac* frameshift mutation in *Escherichia coli* are presented here. I report that, first, the genetic requirements are identical to those for the early steps of homologous recombination in the RecBC(D) pathway; *recA* and *recB* mutants are deficient in recombination and adaptive mutation and a *recD* mutant is hyper-recombinagenic and hypermutable. Second, the parallel genetic requirements do not extend to the late steps of RecBC(D)-mediated recombination which require either the Ruv- or the RecG Holliday junction resolution system; *ruv* mutants are deficient in adaptive mutation, whereas a *recG* mutant is hypermutable, and a transient *ruv recG*-deficiency causes hypermutation. None of these *rec* or *ruv* functions affect growth-dependent Lac reversion. Together, these results suggest that RecBC(D)-mediated recombination intermediates promote adaptive mutation. Third, a strain carrying an antimutator allele of *dnaE* displays decreased mutation. This implies that *dnaE*-encoded DNA polymerase III performs DNA synthesis that can result in adaptive mutations. Fourth, adaptive revertants are not heritably mismatch repair defective. Yet, finally, overproduction of the mismatch repair protein MutL diminishes adaptive but

not growth-dependent mutation. This suggests that MutL, or a protein with which it associates, becomes limiting specifically during adaptive mutation.

These data define components of a novel mutational molecular mechanism that includes homologous recombination, DNA synthesis, and transiently decreased mismatch repair. This mechanism creates mutations, apparently in response to stress. This and similar mechanisms may be important in evolution, development, carcinogenesis, and mutation to drug-resistance and pathogenicity.

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CHAPTER 1

GENERAL INTRODUCTION

Mutations are a primary source of variability that fuels evolution. Mutations also underlie genetic disease, notably carcinogenesis, and provide important tools for genetical studies of biological processes.

Many different routes to mutation are likely to occur (Drake, 1991; Smith, 1992 and references therein). Understanding the multiple molecular mechanisms of mutagenesis is essential for determining how, and how much, each contributes to evolution, developmental change, and human genetic disease.

This introduction will review classical experiments that demonstrated the spontaneous origin of some mutations, and will contrast them with subsequent experiments that suggested the existence of a class of mutations that occur more often when useful. The results presented in this thesis will then distinguish the latter from the former class of mutations in one assay system by elucidating a molecular mechanism by which the latter class form in *Escherichia coli*.

CLASSICAL EXPERIMENTS THAT DEMONSTRATE THE SPONTANEOUS NATURE OF SOME MUTATIONS

The Luria-Delbrück Experiment Salvadore Luria and Max Delbrück provided the first demonstration that some mutations form spontaneously (Luria and Delbrück, 1943). They knew that *E. coli* spread on plates seeded with bacteriophage are mostly killed, but a few phage-resistant mutants survive. They distinguished between two models for the origin of phage T1-resistant mutants. First, the mutants might arise spontaneously at any time during growth of the cultures, before plating with phage. If so, the numbers of mutants in independent cultures would form a highly variable distribution. Cultures in which the first mutation occurred early would contain large numbers of clones of the original mutant (*i.e.* jackpots), whereas cultures in which the first mutation occurred late or not at all would contain few or no mutants. Alternatively, exposure to T1 might induce T1-resistance (*i.e.* mutations occur after exposure to selection). If so, the numbers of mutants in independent cultures would show little variance and fit the Poisson distribution. In multiple experiments with as many as 100 independent cultures, Luria and Delbrück observed a large variance in the distribution of T1-resistant mutants in support of the idea that the mutants arose independently of exposure to the genetic selection.

The results of Luria and Delbrück were not generally accepted (*e.g.* Dean and Hinshelwood, 1966, pp.332-360 and references therein) until confirmation was provided by the experiments of Howard Newcombe and of Joshua and Esther Lederberg.

Newcombe's Experiment Newcombe (1949) realized that the results of Luria and Delbrück were indirect. To find direct evidence for the spontaneous mutation hypothesis, Newcombe took advantage of the fact that a bacterium immobilized on agar forms a colony consisting only of its clones. Aliquots of a T1-sensitive cultures were spread onto two identical sets of agar plates. After several generations of growth, the bacteria on one of the sets of plates were redistributed over the agar's surface with saline solution. Then the cells of both sets of plates were sprayed with T1 and incubated further to allow colony formation. If T1-resistant mutants truly arise during growth of the cells prior to exposure to T1 (*i.e.* spontaneously), then more T1-resistant colonies would be observed on the plates containing redistributed clones of the original cells. However, if mutants arise with a determined probability after contact with the T1, then identical numbers of T1-resistant colonies would be observed regardless of redistribution because both sets contained the same number of cells. Up to 50-fold more T1-resistant mutants were detected on plates with redistributed cells providing direct evidence for a spontaneous origin of mutants.

Replica-plating Experiments of the Lederbergs The development of replica plating by the Lederbergs (1952) provided a way to show the existence of mutants in cells never exposed to selection. Velveteens placed on a wood block with a diameter slightly smaller than an agar plate's surface were used to take imprints of colonies which could then be transferred to other plates. This allows mutant isolation without exposing the original colony to the selective condition. The Lederbergs confirmed and extended the results of Luria and Delbrück by replica-plating large numbers of colonies grown nonselectively to plates seeded with T1 and also to plates containing streptomycin. The distributions of T1- and streptomycin-resistant mutants were highly variable. Thus, in the absence of cells ever experiencing selective conditions, mutations occur. In addition to removing the possibility that mutation to T1- or streptomycin- resistance occurs in response to the selection, these results generalized the conclusions of Luria and Delbrück to include mutation to drug-resistance.

The demonstrations of spontaneous mutation (Luria and Delbrück, 1943; Newcombe, 1949; Lederberg and Lederberg, 1952) support a Darwinian view of evolution in which speciation results from selective forces operating on preexisting genetic variability in a population (Darwin, 1859; reviewed by Fitch, 1982). An opposing view (Lamarck, reviewed by Steele, 1981, pp.7-12) contends that the selective forces induce the heritable changes that allow survival. Thus, the convincing demonstrations that some mutations occur spontaneously have been touted as the death of Lamarckism (*e.g.* Fitch, 1982; Opadia-Kadima, 1987; Gillis, 1991; Keller, 1992).

A POSSIBILITY NOT ADDRESSED BY THE CLASSICAL EXPERIMENTS

Max Delbrück pointed out a limitation in his and others' experiments during the discussion of a paper by André Lwoff at Cold Spring Harbor in 1946 (Lwoff, 1946, p.154):

> "... with respect to the use of the word 'spontaneous' as applying to mutations. The word merely means that certain obvious factors which might be suspected of causing the mutations were shown to be without effect. In bacterial mutations one generally has to use a specific environment which is sharply selective for the mutant. This environment will permit the mutant to come to the foreground. In the case of mutations of bacteria from phage-sensitivity to phage-resistance ... it could be shown that the phage does not cause the mutations (Luria and Delbrück, 1943). (However,) in your (Lwoff's) case of mutations permitting the mutants to utilize succinate, ... it seems to me an obvious question to ask whether this particular medium had an influence on mutation rate, and as long as this has not been ruled out the designation 'spontaneous' would seem improper. In view of our ignorance of the causes and mechanisms of mutations, one should keep in mind the possible occurrence of specifically induced adaptive mutations ..." (parenthetical insertions are mine).

Delbrück appeared to appreciate the fact that the selection with phage T1 was lethal. Phage T1 kills cells within 20 minutes of infection (Hayes, 1968). Streptomycin also kills. Therefore, these selections could not have caused the mutations. Thus, despite the elegance and significance of the classical experiments, the question of whether mutations can also occur in response to a selective environment was not addressed.

EXPERIMENTS THAT SUGGEST THE EXISTENCE OF ADAPTIVE MUTATIONS

Others also realized that the classical experiments did not provide a chance for mutants to arise in response to the selection, notably Francis Ryan (1952b; 1955; Ryan and Wainwright, 1954; reviewed by Ravin, 1976) and about 3 decades later, John Cairns (Cairns *et al.*, 1988; reviewed by Foster, 1993). Their experiments employed non-lethal genetic selections for mutations that are expressed immediately and have revealed mutations that (i) occur in the absence of apparent cell division in a time-dependent manner, (ii) appear to arise only in the presence of a non-lethal genetic selection, and (iii) for many years, were detected only in the gene whose function was selected (*e.g.* Ryan, 1955; Shapiro, 1984; Cairns *et al.*, 1988; Hall, 1988; 1990; 1991; 1992; Cairns and Foster, 1991; Steele and Jinks-Robertson, 1992; reviewed by Foster, 1993; but see Foster, 1997; Hall, 1990; Torkelson *et al.*, 1997; see also Novick and Szilard for an early discovery of generation-independent mutation in slowly growing cells (1950; 1951). These unique mutations are referred to here as adaptive mutations, and the process of their formation as adaptive mutation[1].

Ryan's Experiments Ryan examined mutations disabling a cell's ability to synthesize an amino acid or ferment a sugar (reviewed by Ravin, 1976). He discovered generation-independent mutations apparently induced by selection. Two examples follow.

Concurrently with the Lederbergs' finding (Lederberg and Lederberg, 1952), Ryan reported that a Luria-Delbrück distribution of mutants (Lea and Coulson, 1949; Luria and

---

[1] Such mutations have also been called "stationary-phase" (Ryan and Wainwright, 1954), "directed" (Cairns *et al.*, 1988), "anticipatory" (Hall, 1988; Symonds, 1989), substrate-induced (Davis, 1989), "Cairnsian" (Hall, 1990), "selection-induced" (Hall, 1992), "starvation-associated" (Bridges, 1994), "stressful lifestyle-associated" (Rosenberg, 1994), and "post-plating" (Jayerman, 1995). The term adaptive (Cairns and Foster, 1991) is used here because it is the most common in the literature (see Foster, 1993) and because of the historical precedent (Lwoff, 1946, p.154).

Delbrück, 1943) was not observed in experiments in which the selection was non-lethal. Replicate cultures of *E. coli* unable to metabolize lactose (Lac$^-$) spread on plates containing lactose as the sole carbon source produced a Poisson distribution of mutants on plates[2] (Ryan, 1952b). Although this observation could have been interpreted as evidence for mutational models in which selection plays a role (and was by some, *e.g.* Cairns *et al.*, 1988), Ryan attributed this deviation from the Luria-Delbrück distribution to artifact (Ryan, 1952b).

Studies on reversion of a histidine auxotrophy in *E. coli* revealed a second example of mutation in apparent conflict with the classical studies (Ryan, 1955; Ryan and Wainwright, 1954). Stationary-phase cultures of a histidine auxotroph (His$^-$) produced "adaptive outgrowths" of prototrophic (His$^+$) cells for a period of about 10 days (Ryan and Wainwright, 1954). These outgrowths were due to His$^+$ bacteria that appeared after the His$^-$ cells were diluted in histidineless broth. Thus, they reasoned (cautiously, in a footnote) that this could be due to mutation in stationary phase (Ryan and Wainwright, 1954, p.372).

Extending this observation with large-scale, quantitative experiments, Ryan showed that His$^-$ *E. coli* plated on minimal medium lacking histidine produced late-arising His$^+$ revertants continually over time (Ryan, 1955). The increase in mutation could not be attributed simply to growth-dependent mutation, as no apparent growth of the His$^-$ cells on the plates was detected. Additional trivial explanations for the occurrence of late-arising His$^+$ colonies, such as phenotypic lag (the time between the formation of a mutation and the appearance of its phenotype), slow growth, and cryptic cannibalization, were also ruled out (Ryan, 1957; 1959). An analysis of the variance of the mean numbers of early- and late-arising His$^+$ colonies revealed that the variance of the latter class was much smaller than that of the former. Ryan concluded that the late-arising His$^+$ mutants "had their origin among the non-dividing (His$^-$) bacteria on the (histidineless) plate" (Ryan, 1955, p.729), whereas the early-arising His$^+$ mutants occurred during growth.

---

[2] In a previous study, a different Lac$^-$ *E. coli* strain produced a near-perfect Luria-Delbrück distribution of Lac$^+$ mutants (Ryan, 1952a). Interestingly, this strain was recombination-deficient.

To explain the occurrence of stationary-phase mutation to His$^+$, Ryan invoked DNA turnover, *i.e.* breakdown and re-synthesis during which DNA polymerase errors could produce His$^+$ mutation (Ryan, 1959; Ryan *et al.*, 1959). He found that DNA synthesis could be detected in non-dividing *E. coli* (Nakada and Ryan, 1961). Incorporation of heavy isotopes into the DNA-containing cell fraction could be detected in histidine-starved, stationary-phase cells, and the DNA synthesis that occurred was semiconservative (Nakada and Ryan, 1961).

But is there enough stationary-phase DNA synthesis to account for the observed frequency of His$^+$ mutation? First, to determine whether a positive correlation exists between DNA synthesis and His$^+$ mutation, DNA synthesis, His$^+$ mutation frequency, and cell viability of starved His$^-$ *E. coli* were monitored for 50 hours after histidine deprivation (Ryan *et al.*, 1961). Although a 25% increase in DNA content occurred during the first 5 hours of this experiment, DNA synthesis decreased as the frequency of His$^+$ mutation per viable cell increased. An inverse correlation was observed. Second, based on the assumption that stationary-phase DNA synthesis errors occur at a rate similar to that observed during growth, Ryan *et al.* (1961) estimated that 50% of DNA isolated from 16 day-old, histidine-deprived cultures would have to be replicated. However, less than 5% replicated DNA was detected. Thus, Ryan concluded that stationary-phase mutation to His$^+$, if due to DNA synthesis errors, was due to a small amount of DNA replication in which "errors occur with an unusually high frequency" (Ryan *et al.*, 1961, p.41).

Thus, the mutations found by Ryan--(i) were shown to occur in stationary-phase *E. coli* in the absence of detectable cell division; (ii) were not easily attributable to artifacts such as slow growth, phenotypic lag, or cell turnover; and (iii) appeared not to be due to

DNA synthesis like that occurring during growth. Ryan's stationary-phase His$^+$ mutations appear adaptive in the sense that they permit growth of the mutant on the selective medium. However, it was not shown that accumulation of His$^+$ mutations does not occur in the absence of selection. This would be necessary to conclude that these mutations are truly adaptive.

Ryan's discoveries seemed to have gone overlooked. Had Ryan also looked amongst the cells starved on the histidineless plates for mutations at unselected loci, he may have rekindled the Lamarck *versus* Darwin debate (pondered by Stahl, 1990). The spontaneous mutation hypothesis became dogma and was not challenged again seriously until 1988, 25 years after Ryan's death.

A Fresh Look at an Old Question: The (Re)Discovery of Adaptive Mutation by Cairns, Overbaugh, and Miller In 1988 John Cairns and colleagues published a paper that again questioned the origin of mutants (Cairns *et al.*, 1988). Cairns *et al.* sought to distinguish how much mutation occurs before and after selection with the use of non-lethal genetic selections. Three separate assay systems were used.

First, Cairns *et al.* (1988) examined the mutability of a *lac*Z amber allele [*lacZ*(Am)] located on an F' episome in an *E. coli* strain harboring deletions of the *pro-lac* and *uvrB-bio* chromosomal regions. When spread on plates with lactose as the only carbon source, Lac$^+$ mutant colonies formed after 48 hours of incubation. The distribution of Lac$^+$ mutants amongst several independent cultures was not like that of Luria and Delbrück, nor Poisson, but some combination thereof[3]. This, they suggested, could be due to the presence of two classes of mutations: those that occur in growing cells (early-arising) and those that occur on the plate in stationary-phase cells (late-arising). This idea was supported by their observation that some Lac$^+$ colonies arose after the appearance of obvious growth-dependent jackpots of mutants. These results are similar to those of Ryan (1952b; 1955; Ryan and Wainwright, 1954). However, Cairns *et al.* offered two pieces of evidence that bear on whether the late-arising Lac$^+$ mutations are specific to the selection. First, they showed that late-arising Lac$^+$ mutants did not accumulate when lactose was absent from plates harboring starved Lac$^-$ cells. Second, Cairns and colleagues observed no concomitant accumulation of unselected valine-resistant (Val^r) mutants. Thus, Cairns *et al.* suggested that the mutations might be directed specifically to the gene whose function was selected.

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[3] The *uvr*$^+$ parent of this strain yielded a similar but slightly more Luria-Delbrück-like distribution of Lac$^+$ mutants. Cairns' group also noted that the *uvr*$^+$ parent produced 3- to 5- fold fewer late-arising Lac$^+$ mutants and that the absence of these mutants increased the variance of the distribution (*i.e.* made it more like that observed by Luria and Delbrück). Because this was not what Cairns *et al.* sought to observe, they studied the *uvrB* strain.

Second, Cairns and colleagues used an assay system characterized originally by Shapiro (1984). The assay employs a strain in which DNA between *araB* and *lacZ* is replaced by bacteriophage Mu DNA (Casadaban, 1976). This strain cannot metabolize lactose or arabinose [designated Lac(Ara)$^-$] and must be propagated at ≤32°C to maintain the lysogenic state. Deletion of Mu fuses *araB* with *lacZ* and allows cells to grow on medium containing lactose, provided arabinose is also present to induce transcription of the fusion gene (Shapiro, 1984). Mutations to Lac(Ara)$^+$ occur frequently and for many days in Lac(Ara)$^-$ cells starved at 30°C on minimal lactose-arabinose (MLA) plates (Shapiro, 1984). Fusion rarely occurs during non-selective growth (Shapiro, 1984). Cairns *et al.* asked whether mutation to Lac(Ara)$^+$ depends on the presence of arabinose and lactose in the medium and found that Lac(Ara)$^+$ mutants did not accumulate in the absence of arabinose (also demonstrated by Shapiro, 1984) or lactose, suggesting that Mu excision might be another case of adaptive mutation (Cairns *et al.*, 1988).

Finally, Cairns *et al.* noted that *E. coli* deleted for *lacZ* (*Δlac*), and therefore unable to hydrolyze β-galactosides such as lactose, can activate a cryptic gene, *ebgA*, to do so (reviewed by Hall, 1982). Activation of *ebgA* requires two mutations, one that inactivates *ebgR*, which encodes a repressor of *ebgA*, and another in *ebgA* itself, which encodes a β-galactosidase that can hydrolyze lactose when mutant (Hall and Clarke, 1977). Each mutation occurs randomly during growth at a frequency of less than 10^{-8} (Hall, 1982). If the two mutations occurred independently during starvation and at the same frequency as during growth, then *Δlac E. coli* should form colonies on lactose-containing plates by the *ebg*-dependent route at a frequency of 10^{-16} (Cairns *et al.*, 1988). However, Hall (1982) showed previously that this strain mutates to Lac$^+$ at the high frequency of 10^{-8}. Cairns and colleagues suggested this as a third example of adaptive mutation.

A primary contribution of the Cairns *et al.* paper (1988) was to illustrate that Luria and Delbrück's conclusions might not be exclusive. The new mutagenic mode(s) described by Cairns *et al.* led them to venture "that populations of bacteria, in stationary phase, have some way of producing (or selectively retaining) only the most appropriate mutations" (Cairns *et al.*, 1988, p.144). This implication that some mutations may be purposeful (Lamarckian) provoked debate (Cairns, 1988; Stahl, 1988; Van Valen, 1988; Lenski *et al.*, 1989), applause (Benson, 1988; Symonds, 1989), criticism (Charlesworth *et al.*, 1988; Danchin, 1988; Holliday and Rosenberger, 1988; Partridge and Morgan, 1988; Tessman, 1988), and, importantly, further experimentation.

Criticism Directed at Cairns' Paper The heretical suggestions of Cairns *et al.* provoked criticism. In experiments with the *lac*Z(Am) strain, first, the slight deviation of the colony distributions from random could have been due to slower growth or death of Lac$^+$ mutants before selective plating (Partridge and Morgan, 1988; Tessman, 1988; Lenski *et al.*, 1989), or even to experimental conditions (noted by Cairns *et al.*, 1988; also see Koch, 1982). Second, the late occurrence of Lac$^+$ mutants could be attributed to normal growth-dependent mutation in dividing cells on the lactose plates (Partridge and Morgan, 1988), or to slow growth (*i.e.* a lag in the appearance, Holliday and Rosenberger, 1988) or decreased fitness (Charlesworth *et al.*, 1988) of preexisting Lac$^+$ mutants. Recently, most of the late-arising Lac$^+$ mutants have been found to display slow growth and, therefore, probably exist prior to plating (Prival and Cebula, 1996). Third, the screen for unselected mutations to Val^r amongst the starved Lac$^-$ cells may have been unfair because Val^r mutations are mostly frameshift mutations (Danchin, 1988) which may occur by another mutational pathway and, therefore, may not be comparable to the missense mutations required to revert or suppress the *lac* amber mutation. Also, valine-resistant mutants may be less likely to survive in the absence of valine (Partridge and Morgan, 1988). Thus, the *lac* amber reversion experiments do not demonstrate adaptive mutation.

The apparent adaptiveness (Cairns *et al.*, 1988) of the Mu excision system of Shapiro (Shapiro, 1984) has been debated (Partridge and Morgan, 1988; Cairns, 1990; Mittler and Lenski, 1990a; Mittler and Lenski, 1990b). Partridge and Morgan (1988) pointed out that these experiments lack a control for generalized excision of Mu under the experimental conditions. Could starvation on MLA plates stimulate Mu excision at other loci? Mittler and Lenski (1990b) showed that starvation *per se* can stimulate Mu excision and argued that the rate of Mu excision to Lac(Ara)$^+$ is similar on M as on MLA plates. This result is opposite to that of Cairns *et al.* (1988) who detected no excision of Mu in rich medium lacking lactose or arabinose. One difference between these two sets of experiments is the media - minimal *vs* rich. However, Cairns noted that Mu excision did not occur in starved cells even on minimal plates (Cairns, 1990). Yet Shapiro himself could obtain results similar to Mittler and Lenski (personal communication in Mittler and Lenski, 1990a, reported by Maenhaut-Michel and Shapiro, 1994)[4]. Eventually, Cairns concurred (Foster and Cairns, 1994). However, although Mu excision is not selection-specific, the DNA sequences of the excision-junctions that form during selection differ from those occurring non-selectively (Maenhaut-Michel and Shapiro, 1994) supporting the possibility that a different mechanism operates under selection. That Mu excises in response to stress is similar to Barbara McClintock's findings of environmentally-induced transposition in maize (McClintock, 1978; 1984).

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[4] Note that this conflicts with Shapiro's original observation that fusions failed to occur in the absence of arabinose (Shapiro, 1984).

Nearly every aspect of the data of Cairns *et al.* (1988) has been criticized and a key component has been disproved (Prival and Cebula, 1996). Nevertheless, the paper by Cairns, Overbaugh, and Miller (1988) fulfilled its primary purpose to "show how insecure is our belief in the spontaneity of most mutations" and, most importantly, catalyzed further productive reinvestigation into the origin of mutants (see below).

Adaptive Mutation at Other Loci in *E. coli*: Studies by Hall Shortly after publication of Cairns' article, Hall described two additional examples of adaptive mutation (Hall, 1988; 1990; 1991; see Foster, 1993, for a review of these and other possible examples).

The *bgl* operon of *E. coli* is normally not expressed but when activated allows utilization of β-glucosides such as salicin (Hall, 1988). Activation requires two mutations: (i) a point mutation, $bglR^0 \rightarrow bglR^+$, which allows transcription of the structural genes required for salicin utilization; and (ii) an IS element excision, *bglF::*IS*103* → $bglF^+$, which restores function to the gene whose product transports salicin into the cell. Hall scored numbers of Sal^+ papillae occurring on starved Sal^- colonies on MacConkey-salicin plates in which carbon is limiting such that colonies reach a maximum of about 10^9 cells (Hall, 1988). The frequencies of growth-dependent mutation to $bglR^+$ and $bglF^+$ are 6×10^{-8} and $\sim 2 \times 10^{-10}$ (mutations per cell), respectively (Parker *et al.*, 1988). Therefore, the probability of producing one Sal^+ papilla should be about 10^{-18}. However, in 14 days, more than 50% of the Sal^- colonies produced Sal^+ papillae. These events were not detected in the absence of salicin and the mutation rate to valine resistance was not elevated in the salicin-starved colonies. Hall also showed that IS excision, but not mutation to *bglR*$^+$, could be detected prior to the appearance of any Sal$^+$ papillae. However, unlike starvation-stimulated Mu excisions (above), excision of IS*103* was not detected in the absence of selection and may not be a general response to starvation. This observation suggested to Hall that cells anticipate and then generate the mutations that permit growth, even though IS excision alone confers no obvious selective advantage (also discussed by Symonds, 1989). These experiments are subject to many of the same criticisms that Cairns *et al.* received (above).

Second, studying reversion of tryptophan auxotrophs (Trp$^-$) to prototrophy (Trp$^+$) on medium with limiting amounts of tryptophan, Hall found that Trp$^+$ mutants are detected as papillae on Trp$^-$ colonies containing about 5 x 10^7 cells (Hall, 1990; 1991). Reversion frequencies (mutations per cell) of strains carrying point mutations in *trpA* and *trpB*, which encode essential subunits of tryptophan synthetase, are much higher under selective conditions than during growth under non-selective conditions (Hall, 1990). Trp$^+$ papillae accumulated with time, formed a distribution similar to the Poisson, and did not occur in colonies starved for another amino acid, cysteine (similarly, Cys$^+$ mutants did not occur in colonies starved for tryptophan). Cryptic growth, and therefore normal growth-dependent mutation, did not occur detectably in the old, Trp$^-$ colonies. Also, other artifacts such as phenotypic lag of preexisting Trp$^+$ mutants could be eliminated. Starvation for tryptophan appeared not to be generally mutagenic as increased rates of mutation to Val^r and *lacI*$^-$ were not detected in starved colonies. Thus, mutation to Trp$^+$ appears to be adaptive.

Interestingly, 2 auxotrophs were found amongst 110 Trp$^+$ mutants, a frequency at least 18 times higher than amongst other starved cells (Hall, 1990). To explain this observation, Hall proposed that a fraction of starved cells enters a hypermutable state which kills them unless they are rescued by mutation to Trp$^+$ (Hall, 1990). This model accounts for the high frequency and the apparent gene-specificity of adaptive mutation (Hall, 1990; discussed further in CHAPTER 7; see also Torkelson *et al.*, 1997).

In a subsequent study, Hall showed that a *trpA trpB* double mutant reverts to Trp$^+$ 10^8-fold more frequently than would be predicted if the two mutations resulted from random, independent events (Hall, 1991). Does this result support Hall's hypermutable state model (Hall, 1990)? Hall estimated that a hypermutated cell would contain 4 mutations per 100 base pairs, a number not supported by DNA sequencing (Hall, 1991). This caused rejection of the hypermutable state model (Hall, 1991). However, Hall later realized that rejection may have been premature because crossfeeding by single-mutant intermediates had not been considered (Hall, 1993). Moreover, the kinetics of reversion of the single mutants, *trpA* and *trpB*, to Trp$^+$ are first order, whereas the kinetics of reversion of the double mutant are second order, as if they were the product of the kinetics of the single mutants (*i.e.* as if each mutation is an independent event occurring in the same hypermutable cell).

In summary, Hall's data imply that adaptive mutagenesis occurs not just in catabolic genes (*e.g. lac*), but also in anabolic genes (*e.g. trp*). His data highlight the differences between mutations caused by mobile elements and those caused by base substitutions. Hall's hypermutable state model can explain the existing data in Darwinian terms (see CHAPTER 7 for further discussion; compare also with other models for adaptive mutation by Stahl, 1988; 1990; 1992; Davis, 1989; Foster, 1992; Foster and Cairns, 1992).

Adaptive Mutation in Yeast[5] That eukaryotes undergo adaptive mutation has been supported by studies of late-arising revertants of a yeast *lys2* frameshift mutation (Steele and Jinks-Robertson, 1992), a *his4* missense mutation (Hall, 1992), and a *his6* missense mutation (Hall, 1992). All were found to occur only in the presence of the appropriate genetic selection, were not accountable by artifacts such as cell turnover, cryptic growth, crossfeeding, or phenotypic lag, and formed distributions that appear to be Poisson (for *lys2*)[6]. Furthermore, elevated frequencies of an unselected mutation (resistance to inositol) were not observed in starved *his4* cells (Hall, 1992). These data imply that yeast can accrue adaptive mutations similar to those in *E. coli*.

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[5] An early hint of adaptive mutation in yeast is von Borstel's observation that a tryptophan auxotroph (*trp1-1*) continually produced Trp$^+$ revertants during selection (von Borstel, 1978). However, it was not determined whether the Trp$^+$ revertants were selection-specific.

The *lac* Frameshift Assay System, described by Cairns and Foster (1991), uses an *E. coli* strain carrying a chromosomal *lac* deletion and an episomal (F') *lac* frameshift mutation (CCC to CCCC) in the *lacI* portion of a constitutively transcribed *lacI-lacZ* fusion gene (Cairns and Foster, 1991). The *lac* frameshift-bearing cells are spread onto minimal lactose plates with a 10-fold excess of nonrevertable, Lac$^-$ "scavenger" cells that also contain an F' episome (Cairns and Foster, 1991). Scavenger cells consume any non-lactose carbon sources that might be present in the medium. Growth-dependent Lac$^+$ revertants arise early, after two days of incubation at 37°C. Adaptive Lac$^+$ revertants arise later and continuously over time (see Cairns and Foster, 1991; CHAPTER 2, FIGURE 2-1)[7]. Under these conditions, little change in the numbers of viable *lac* frameshift-bearing cells occurs (see Cairns and Foster, 1991; CHAPTER 2, FIGURE 2-1). After 5 or 6 days of incubation, 90% or more of the observed Lac$^+$ revertants occur in the absence of net cell growth, suggesting that the majority of Lac$^+$ mutation in this assay system is time-dependent, not growth-dependent. The distributions of early- *versus* late-arising Lac$^+$ mutants also support this view (Cairns and Foster, 1991). As with other assay systems, late-arising mutants fail to accumulate in the absence of selection (lactose, Cairns and Foster, 1991) and an unselected mutation (*rpoB*, Foster, 1994) was not detected amongst starved Lac$^-$ cells (but see Foster, 1997; Torkelson *et al.*, 1997, discussed in CHAPTER 7). The robust adaptive response of the *lac* frameshift-bearing strain (see Cairns and Foster, 1991; CHAPTER 2, FIGURE 2-1) makes this assay system particularly amenable to genetic analyses. It is used in all of the experiments described in this thesis.

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[6] Hall was unable to examine distributions of the His$^+$ mutants as a significant proportion were second-site suppressors (Hall, 1992). These mutants grow more slowly than true revertants and make it difficult to determine adaptive contributions to mutant distributions.

[7] Jackpots of growth-dependent Lac$^+$ mutants are excluded from calculations determining the number of Lac$^+$ colonies per 10^8 viable cells plated. A culture is considered a jackpot if it contains a number of mutants greater than two standard deviations above the mean number of mutants calculated without that culture.

THESIS OBJECTIVES AND ADDITIONAL BACKGROUND

The objective of this thesis is to elucidate a molecular mechanism of adaptive mutation in *E. coli*. If the molecular mechanism of adaptive mutation is different from that of growth-dependent mutation then the two must be different processes. The data in this thesis offer such a distinction.

Hastings and Rosenberg imagined that non-dividing and, perhaps, non-replicating cells might build a functional gene by recombining the inactive gene with partially identical (homeologous) DNA from other regions of the genome (Hastings and Rosenberg, 1992; see Cairns *et al.*, 1988; Foster, 1992; Foster and Cairns, 1992; Grafen, 1988; Stahl, 1992; Thaler, 1994; Thaler *et al.*, 1990 for other recombinational models). Somatic hypermutation of chicken immunoglobulin genes occurs by such a mechanism (Maizels, 1987; Maizels, 1995). In chickens, the V genes, which encode the antigen binding domains of the immunoglobulins, recombine with homeologous pseudo-V genes located elsewhere. To test recombinational models for adaptive mutation in *E. coli*, we asked whether recombination proteins are required for Lac$^+$ adaptive reversion (CHAPTER 2). A summary of those proteins in the context of a probable model (Rosenberg and Hastings, 1991) follows:

Homologous recombination in *E. coli* RecBCD pathway (reviewed by Clark and Sandler, 1994; Myers and Stahl, 1994) begins with a DNA double-strand break (DSB). RecBCD enzyme loads onto double-strand DNA (dsDNA) at a DSB and degrades the DNA until it encounters a Chi sequence (χ). At χ the RecD subunit, which is required for nuclease activity, is inactivated and the RecBC(D$^-$) enzyme unwinds the dsDNA, creating recombinagenic single-strand DNA (ssDNA) ends. ssDNA is coated with RecA protein and invades a homologous dsDNA molecule to produce a heteroduplex recombination intermediate. Resolution of such recombination intermediates occurs by either a RuvABC-dependent route or a RecG-dependent route. Essential for this pathway are DSBs, RecBC(D), RecA, and either RuvABC or RecG (reviewed by Rosenberg and Hastings, 1991; Myers and Stahl, 1994; West, 1994).

In support of recombinational models for adaptive mutation, Cairns and Foster showed that reversion of the *lac* frameshift mutation is diminished by a mutation in *recA* (Cairns and Foster, 1991). However, RecA also governs the regulation of the SOS response, which includes more than 20 genes that are induced in response to DNA damage (reviewed by Walker, 1996; see also APPENDIX II). The Cairns and Foster study did not distinguish whether RecA acted *via* recombination or SOS in Lac$^+$ adaptive mutation (discussed in detail in APPENDIX II), but did demonstrate that the mechanisms of adaptive- and growth-dependent Lac reversion are distinct in their use of RecA.

Data in CHAPTER 2 (Harris *et al.*, 1994) show that the genetic requirements for Lac$^+$ adaptive mutation parallel those for the early steps of homologous recombination in the RecBC(D) pathway: *recA* and *recB* null mutants are deficient in adaptive mutation and recombination, whereas a *recD* null mutant is hypermutable adaptively and hyper-recombinagenic. None of these *rec* mutations affects growth-dependent Lac reversion (CHAPTER 2). Thus, the molecular mechanism of Lac$^+$ adaptive mutation is distinct from growth-dependent Lac reversion in that it employs RecBC(D)-mediated homologous recombination.

Is resolution of recombination intermediates, also required for Lac$^+$ adaptive mutation? The data in CHAPTER 3 (Harris *et al.*, 1996) indicate that RuvABC-dependent resolution of recombination intermediates is required, but that the recombination intermediates themselves promote Lac$^+$ adaptive mutation: *ruv* null mutants are deficient in Lac$^+$ adaptive mutation, whereas a *recG* null mutant is hypermutable adaptively. A transient *ruv recG*-deficiency causes adaptive hypermutation. None of these mutations affect growth dependent Lac reversion. These data further distinguish adaptive- and growth-dependent mutation in this assay system (see also Foster *et al.*, 1996) and demonstrate a role for recombination intermediates in Lac$^+$ adaptive mutation.

Recombination could produce Lac$^+$ adaptive mutation in two different ways (Harris *et al.*, 1994). Templated mutation models, like the model of Hastings and Rosenberg (1992), predict that only a limited number of mutant sequences will be observed and that additional "hitchhiking" mutations from the template are likely to accompany the mutation conferring Lac$^+$. Non-templated mutation models are also tenable. For example, recombination could prime DNA synthesis that contains errors that become Lac$^+$ adaptive mutations. Non-templated Lac$^+$ mutations would probably not be restricted to a limited number of DNA sequence changes nor be found with "hitchhikers".

In work not in this thesis, these two classes of models were distinguished by sequencing a portion of the *lacI-lacZ* fusion gene of a number of adaptive and growth-dependent Lac$^+$ mutants (Rosenberg *et al.*, 1994). We found, first, that adaptive reversions of the +1 *lac* frameshift mutation are mostly -1 deletions in small mononucleotide repeats (Rosenberg *et al.*, 1994; similar data reported by Foster and Trimarchi, 1994). These mutations are accompanied by no extraneous base changes and are not derived obviously from templates, supporting non-templated models for Lac$^+$

adaptive mutation. Second, in contrast to the adaptive Lac reversions, growth-dependent reversions of the *lac* frameshift mutation are heterogeneous, consisting of insertions, deletions, and more complex events. This demonstrates that adaptive mutations themselves are different. Third, the adaptive Lac reversion sequences are mostly single base deletions in mononucleotide runs. These are characteristic errors of DNA polymerases (Ripley, 1990) that escape mismatch repair (Levinson and Gutman, 1987; Cupples *et al.*, 1990; Strand *et al.*, 1993; Modrich, 1994). These keys led to the following discoveries: (i) we identify a DNA polymerase that makes Lac$^+$ adaptive mutations (CHAPTER 4, Harris *et al.*, 1997a); (ii) we show that adaptive mutants are not heritably mismatch repair-defective (CHAPTER 5; Longerich *et al.*, 1995; Torkelson *et al.*, 1997). This supports the hypothesis that a transient deficiency of mismatch repair occurs during adaptive mutation (see Stahl, 1988; Rayssiguier *et al.*, 1989; Hastings and Rosenberg, 1992; Rosenberg, 1994; Rosenberg *et al.*, 1994; 1995; 1996; Longerich *et al.*, 1995 for hypotheses); and (iii) we provide evidence that mismatch repair function becomes limiting specifically during Lac$^+$ adaptive mutation due to a lack of functional MutL protein (CHAPTER 6, Harris *et al.*, 1997b).

The methyl-directed mismatch repair system of *E. coli* is a key enforcer of genome stability. Mismatch repair prevents recombination of diverged DNA sequences (Radman *et al.*, 1995) and corrects lesions in DNA such as DNA polymerase errors (Modrich, 1991; Modrich, 1995). Absence of mismatch repair causes genetic instability in bacteria, yeast, and humans (Modrich, 1994). In *E. coli*, MutS, MutL, MutH, and MutU proteins are essential for mismatch repair *in vivo* (Schaaper, 1993). Single-strand dependent exonucleases, ExoI, ExoVII, and RecJ, which were hypothesized to be required (Grilley *et al.*, 1993; Modrich, 1995), are found not to be essential for mismatch repair *in vivo* (APPENDIX IV, Harris *et al.*, 1997c).

The data in this thesis define components of a molecular mechanism of adaptive mutation that includes DNA double-strand breaks, homologous recombination, DNA synthesis, and transiently decreased mismatch repair.

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CHAPTER 2

RECOMBINATION IN ADAPTIVE MUTATION*

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Adaptive mutation is a process that appears to produce useful mutations only in the presence of selection for those mutations and in the absence of cell growth (Cairns *et al.*, 1988; Cairns and Foster, 1991; Foster and Cairns, 1992; Foster, 1993). Its molecular mechanism has not yet been elucidated, and because the properties of adaptive mutation challenge established dogma regarding the mechanisms by which mutations occur (Luria and Delbrück, 1943), the reality of adaptive mutation as a phenomenon distinct from spontaneous mutation in growing cells has been questioned (Cairns, 1993; Lenski and Mittler, 1993a; 1993b). In this report, we address the mechanism of adaptive mutation, which distinguishes adaptive mutation from mutation in growing cells. Unlike spontaneous growth-dependent mutations, adaptive reversion of a *lac* frameshift mutation (Cairns and Foster, 1991; Foster and Cairns, 1992) in *Escherichia coli* requires genetic recombination genes of the bacterial RecBCD recombination system. These results indicate that recombination is part of the molecular mechanism by which adaptive mutations occur.

The system used for monitoring adaptive mutation is that described by Cairns and Foster (1991; Foster and Cairns, 1992). Samples of *E. coli* carrying a *lacI-lacZ* fusion gene with a frameshift mutation (*lac*$^-$) are plated on minimal lactose medium. Such cells cannot form colonies unless they mutate to Lac$^+$. The Lac$^+$ mutant colonies arise continuously during exposure to the selective medium (FIGURE 2-1). No net increase occurs in the number of *lac*$^-$ cells that generate the Lac$^+$ mutants. The number of *lac*$^-$ cells is assayed by removing a fixed volume of agar from the plate each day, suspending the cells in liquid, and then assaying the number of *lac*$^-$ viable cells on rich medium (FIGURE 2-1). In all of the experiments reported here, with every recombination-mutant genotype examined, neither net growth nor death of the *lac*$^-$ cells was observed over the course of the experiment. The adaptiveness of the late-arising mutants described here has not been demonstrated by us but relies on previous results (Cairns and Foster, 1991) with the same strain (FIGURE 2-1; Cairns and Foster, 1991) and is not the subject of this study. This study addresses the mechanism by which the late-arising mutants form. The term adaptive mutation, used by (Cairns and Foster, 1991; Foster and Cairns, 1992; Cairns, 1993; Foster, 1993; Lenski and Mittler, 1993a; Lenski and Mittler, 1993b), is used here to identify the phenomenon as that described previously (Cairns and Foster, 1991; Foster and Cairns, 1992; Foster, 1993) rather than as an assertion that we have demonstrated adaptiveness.

To test whether recombination is necessary for adaptive mutation (Cairns *et al.*, 1988; Thaler *et al.*, 1990; Foster and Cairns, 1992; Hastings and Rosenberg, 1992; Thaler, 1994), recombination-defective mutant derivatives of the *lacI-lacZ* frameshift-bearing strain were examined for their ability to mutate adaptively. The primary RecBCD recombination system of *E. coli* requires RecA protein and RecBCD enzyme (Rosenberg and Hastings, 1991). A partial-function, recombination-impaired *recA* point mutant shows decreased adaptive mutation (Cairns and Foster, 1991; FIGURE 2-2-A). In a null *recA* deletion strain, adaptive mutation is abolished (FIGURE 2-2-A and B; Foster, 1993). Because RecA protein functions in processes other than recombination (Devoret and Weinstock, 1991), these results do not exclude nonrecombinational models for the mechanism of adaptive mutation.

RecBCD enzyme is a heteromultimer with subunits encoded by the *recB*, *recC*, and *recD* genes (Taylor, 1988). Null mutations in *recB* or *recC* destroy all function of the enzyme and render cells recombination-deficient. A *recB* null mutant is deficient in adaptive mutation (FIGURE 2-2-A). RecD functions as a negative effector of the recombination activity of RecBCD (Thaler *et al.*, 1989; Rosenberg and Hastings, 1991) such that *recD* null mutants are hyper-recombinagenic (Amundsen *et al.*, 1986; Biek and Cohen, 1986; Thaler *et al.*, 1989). We find that a *recD* null mutant strain is adaptively hypermutable (FIGURE 2-2-A and B).

We tested whether the hypermutation observed in *recD* cells is the result of hyper-recombination. Just as the hyper-recombination in *recD* cells depends on functional *recA*

(Amundsen *et al.*, 1986; Biek and Cohen, 1986), we find that the hypermutation in *recD* also requires functional *recA* (FIGURE 2-3). The hyper-recombination in *recD* mutants is the result of a hyper-recombining RecBC(D$^-$) enzyme, and requires functional *recB* (Amundsen *et al.*, 1986; Biek and Cohen, 1986). Hypermutation in *recD* cells also requires functional *recB* (FIGURE 2-3). Because the hypermutation in *recD* requires genes of the RecBCD recombination pathway, like adaptive mutation in *rec*$^+$, it is unlikely that the *recD* mutation activates a different route to the formation of adaptive mutations. These data indicate that the same pathway is used to form Lac$^+$ revertants in *recD* cells and in *rec*$^+$ cells.

DNA can be recombined by *E. coli* with the use of subsidiary recombination pathways called RecE and RecF, which require different *rec* genes, including *recJ* and *recQ* (Clark and Low, 1988). These genes, which are not necessary for RecBCD-mediated recombination, are also not necessary for adaptive reversion to Lac$^+$ (FIGURE 2-4).

The genetic requirements for adaptive mutation described are identical to those for recombination in the RecBCD system. Such requirements are not observed for spontaneous reversion of the same *lac* frameshift mutation in growing cultures (TABLE 2-1; Cairns and Foster, 1991). These results indicate a mechanistic difference between the two sorts of mutation (Cairns and Foster, 1991; Cairns, 1993; Lenski and Mittler, 1993b), and indicate that recombination is part of the molecular mechanism of adaptive mutation but not part of the mechanism of mutation in growing cells.

Several different recombinational models for the molecular mechanism of adaptive mutation are tenable. All of them can be classified as either templated or nontemplated. In the templated mutation models, the mutant sequence or sequences that restore *lac*$^+$ information preexist in the genome. These sequences are transferred into the mutating gene by recombination or gene conversion (Thaler *et al.*, 1990; Hastings and Rosenberg, 1992; Thaler, 1994). In the nontemplated mutation models, although recombination is required for formation of the adaptive mutation, the mutant sequence is formed *de novo* (Cairns *et al.*, 1988; Foster and Cairns, 1992).

Hastings and Rosenberg (1992) previously suggested a model in which adaptive mutations are templated from partially homologous (homeologous) sequences elsewhere in the genome. The normal barriers to homeologous recombination (Rayssiguier *et al.*, 1989) were proposed to be relaxed during stress, so that homeologies could recombine.

An example of a recombinational mechanism in which mutations are not templated is mutagenic recombination. In yeast mitotic recombination, new mutations are found near recombination junctions [(Strathern *et al.*, 1995); foreshadowed by work in *Salmonella* (Demerec, 1962), yeast (Magni and von Borstel, 1962; Esposito and Bruschi, 1993), and filamentous fungi (Paszewski and Surzycki, 1964)]. Formation of the mutations is thought to result from an error-prone repair synthesis associated with recombination. If this faulty synthesis also occurs in *E. coli*, then any mechanism that increases recombination could increase mutation. Adaptive mutations could result from high-frequency recombination, even between identical molecules (sister chromosomes or intrachromosomal duplicated regions). The sequencing of adaptive mutations will help to distinguish models in which mutations are templated from those in which they are not.

In both templated and nontemplated mutation models, the failure to find selection-induced irrelevant mutations (Cairns *et al.*, 1988) could be achieved by invoking a hypermutable state which a subpopulation of cells enters, and that cells either die from or exit only by generating an adaptive mutation (Foster, 1993). In such models irrelevant genes mutate, but because the cells die if they do not become Lac$^+$, nonadaptive mutations are not observed. Our results suggest the molecular basis of such a state.

The recombination genes necessary for adaptive reversion to Lac$^+$ are those of the RecBCD system. Although RecBCD is used for recombination in conjugation, recombination of the vegetative bacterial chromosome is mostly RecBCD-independent (Mahan and Roth, 1989). This independence can be understood upon consideration that

RecBCD enzyme loads onto DNA at double-strand breaks (DSBs) (Thaler and Stahl, 1988), which should be present during conjugation, but are not expected in the vegetative chromosome. We suggest that during stress, DSBs are created in the bacterial chromosome and allow RecBCD to load, thereby elevating recombination dramatically. For example, 10^5-fold more recombination is seen at the terminus of replication, where DSBs are thought to occur (Louarn *et al.*, 1991). Double-strand ends could form at paused replication forks by annealing the new strand-ends with each other instead of with the old strands (Louarn *et al.*, 1991). Other means of DSB formation are also possible. Double-strand break formation, allowing high-level recombination, could be the molecular basis of the hypermutable state (Foster, 1993) that either kills or is stopped when an adaptive mutation (templated or nontemplated) rescues the cell. Failure to make the adaptive mutation allows continued double-strand breakage, which kills.

Gene duplication or amplification (Foster and Cairns, 1992) could provide a means by which the DSBs postulated here could be repaired. Survivors of selection would be those cells that (i) form a DSB which allows RecBCD loading; (ii) contain a preexisting duplication of the DNA segment in which the DSB forms, which allows recombinational repair of the DSB; and (iii) recombine the *lac* gene with either the identical duplicated *lac* gene or with a partially homologous *lac* region elsewhere to form nontemplated or templated mutations, as described above.

TABLE 2-1. Mutation rates in growing cultures of *rec* mutant strains.

rec genotype	Experiment	Number of cultures	Number of revertants in median culture	Median number of revertants (per 10^{10} cells)	Mutation rate to Lac⁺ (mutations per cell per generation)
rec⁺	1	37	4	42.1	5.7×10^{-10}
	2	59	4	41.2	5.7×10^{-10}
ΔrecA	1	38	1	23.3	3.6×10^{-10}
	2	59	1	7.2	1.5×10^{-10}
recB	1	38	2	117	13×10^{-10}
	2	59	1	43.9	5.9×10^{-10}
recD	1	40	2	25.4	3.9×10^{-10}
	2	59	2	16.9	2.8×10^{-10}

Independent cultures of each strain were grown from single colonies to saturation in minimal (M9, proline, thiamine) 0.1% glycerol liquid medium, washed in the same medium without glycerol, concentrated and plated on minimal lactose (as described in FIGURE 2-1) to measure Lac⁺ revertant colonies, and on rich (LB) plates to assay viable cells. To obtain counts only for mutants that preexisted in the liquid cultures rather than mutants that arise after plating, experiments were performed using two separate Lac⁺ revertants of each *rec* genotype to determine the earliest time after plating that colony counts could be taken accurately on the selective plates. These times (30 hours for *rec*⁺ and *recD*, 36 hours for *recA*, and 40 hours for *recB*) were then used for scoring the presence of Lac⁺ revertants that arose during the growth of the liquid cultures. Mutation rates are calculated by the method of the median (Lea and Coulson, 1949, as modified by von Borstel, 1978).

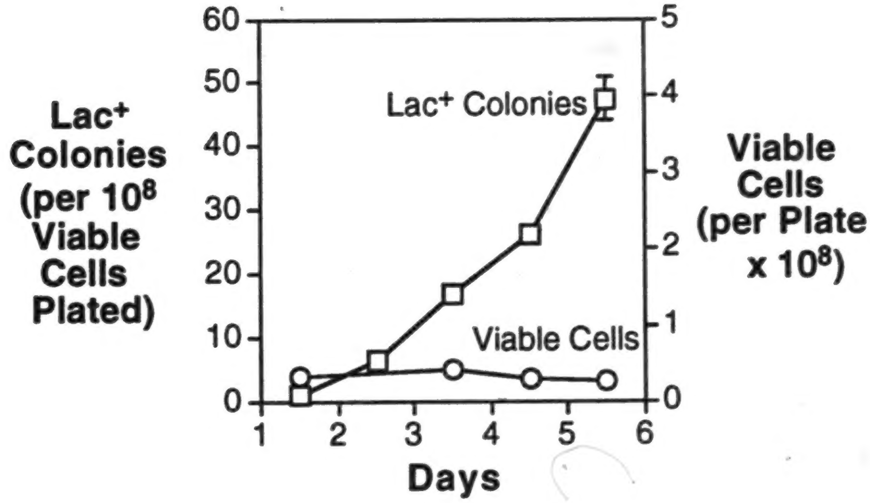
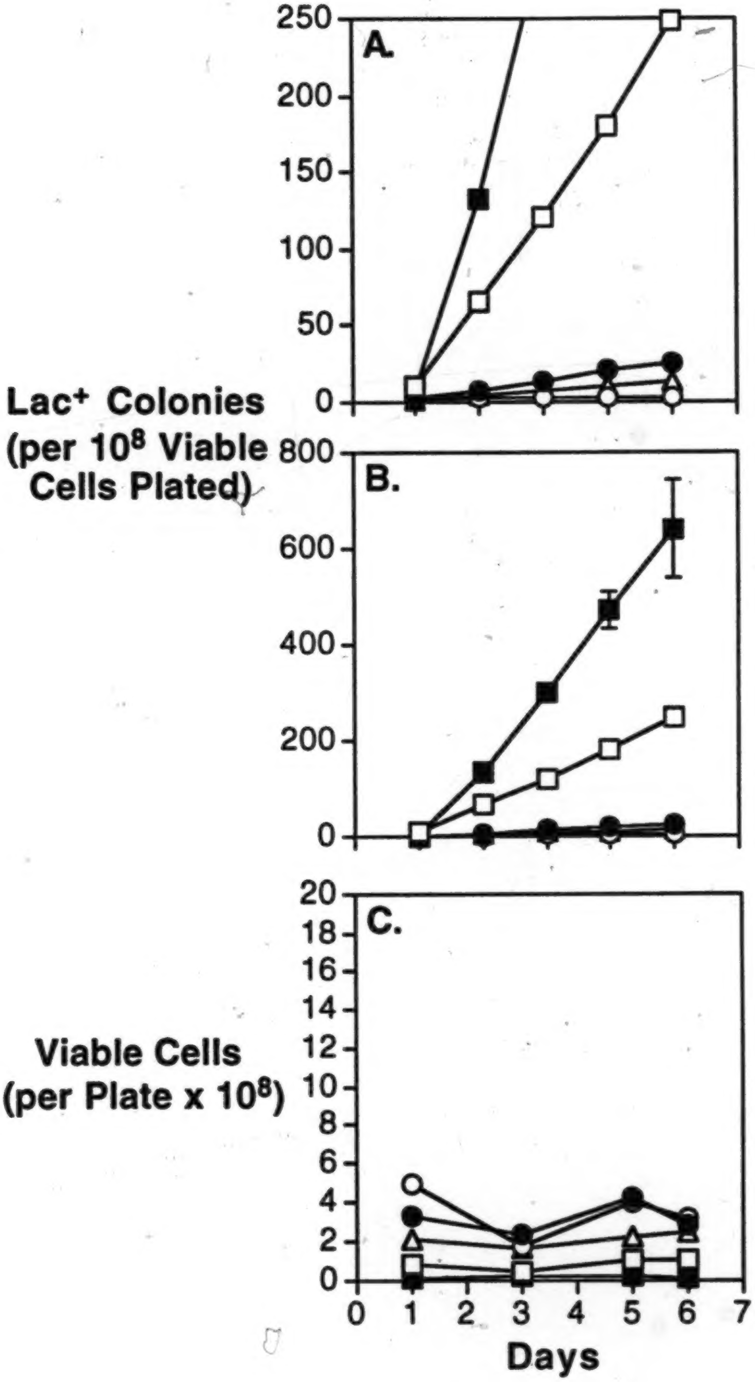


FIGURE 2-1. Adaptive mutation of a *lac* frameshift mutation to Lac+. The *rec+ lacI-lacZ* frameshift-bearing strain (Cairns and Foster, 1991) carries a fusion of the *lacI* and *lacZ* genes which is constitutively transcribed from the *lacI^q* promoter, and which bears a CCC to CCCC frameshift in *lacI* that is polar on *lacZ*. This strain was plated as described by Cairns and Foster (1991) on minimal lactose plates, in the presence of an excess of *Δlac* scavenger cells. Selective plates were overlayed with M9 proline, thiamine, 0.1% lactose top agar containing 10^{10} scavenger cells per plate, incubated for one day at 37°C, and then overlayed with *lac-* frameshift cells plus 10^9 scavenger cells on day 0 of each experiment. This procedure gave consistent inhibition of growth of the frameshift cells on the minimal lactose plates. Medium is as described (Cairns and Foster, 1991) and contained 0.1% lactose. The scavenger cells do not mutate to Lac+ and are added to consume any residual nonlactose carbon sources that may be present. The Lac+ mutant colonies are allowed to accumulate over time. The scavengers are rifampicin-sensitive and the frameshift-bearing strain is rifampicin-resistant. Viable cell counts of the *lac-* frameshift cells were performed (Cairns and Foster, 1991) by removing a plug of agar from between visible Lac+ colonies, suspending in M9 broth, and plating on rich, rifampicin medium (LB or MacConkey lactose) to determine the number of colony-forming units. No net increase of the *lac-* cells was observed during the period when increasing numbers of Lac+ revertant colonies were scored, in agreement with previous reports (Cairns and Foster, 1991; Foster and Cairns, 1992). Error bars represent one standard error of the mean and, when not visible, are smaller than the symbols they bracket. Means were of counts from two cultures plated on six plates plus two cultures plated on five plates (n=22).

FIGURE 2-2. Ability of *rec* mutants to mutate adaptively. Experiments are as in FIGURE 2-1. **A.** Reversion of *lac* in different *rec* mutant derivatives of the *lacI-lacZ* frameshift-bearing strain: *rec*$^+$ (open squares); *recA430* (filled circles), a point mutation (Cairns and Foster, 1991). All other *rec* mutant strains were constructed for this work with the use of standard P1 transduction methods and carry *rec* null alleles: *Δ(srlR-recA)306*::Tn*10* (open circles), a *recA* deletion that confers recombination-deficiency. The *recB21* allele (open triangles), abolishes all function of RecBCD recombination enzyme and thus is also recombination-deficient. The *recD* null allele used here, *recD6001*::Tn*10Kan* (filled squares), was constructed for this work as follows: The *tetA* gene of *recD1903*::Tn*10* was disrupted with a kanamycin-resistance cassette using the method of François *et al.* (1987). A *recD*$^-$, *recBC*$^+$ isolate was screened as a kanamycin-resistant, tetracycline-sensitive strain that permits large plaque formation of phage λ *red gam* Chi°, and is ultraviolet-resistant. Null mutations of *recD* confer a hyper-recombinagenic phenotype (Amundsen *et al.*, 1986; Biek and Cohen, 1986; Rosenberg and Hastings, 1991; Thaler *et al.*, 1989; Thaler and Stahl, 1988). **B.** Data shown in (A) expressed on an expanded ordinate in which *recD* results are visible. For *rec*$^+$, n=24 on all days except 5 and 6, on which n=12; *recA430*, n=18; *ΔrecA*, n=12; *recB*, n=24; *recD*, n=29 except on day 6, on which n=14. **C.** Viable cell measurements of the various *rec* strains over the course of the experiment illustrated in (A) and (B).


Lac⁺ Colonies
(per 10⁸ Viable Cells Plated)
A.
250
200
150
100
50
0
B.
800
600
400
200
0
C.
20
18
16
14
12
10
8
6
4
2
0
Viable Cells
(per Plate x 10⁸)
0
1
2
3
4
5
6
7
Days

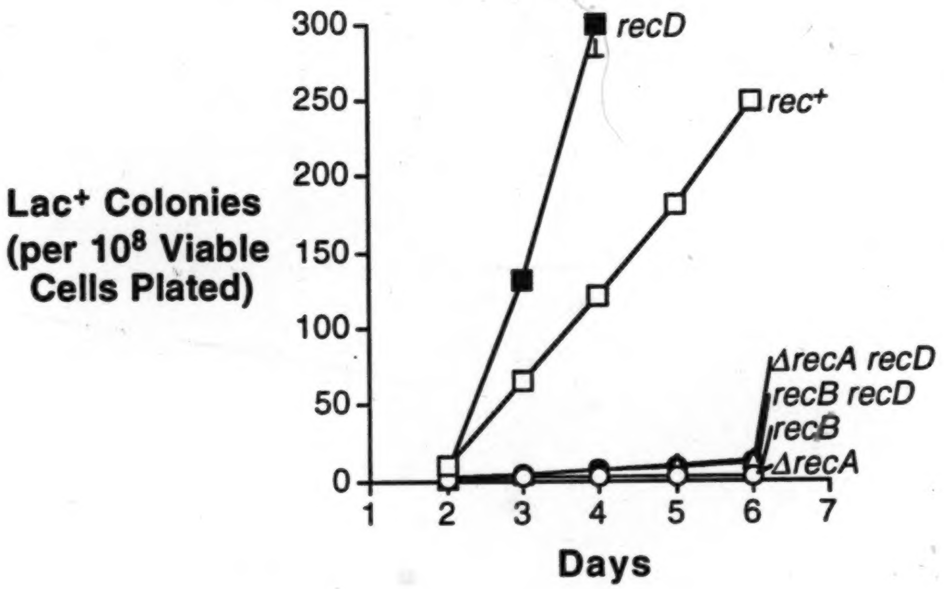


FIGURE 2-3. Functional *recA* and *recB* genes are required for adaptive hypermutation in *recD* mutants. Experiments and data presentation as in FIGURES 2-1 and 2-2. Data and symbols for single *rec* mutants are the same as those shown in FIGURE 2-2. Filled circles, *Δ(srlR-recA)306*::Tn*10* *recD6001*::Tn*10Kan* (n=17). Filled triangles, *recB21* *recD6001*::Tn*10Kan* (n=12).

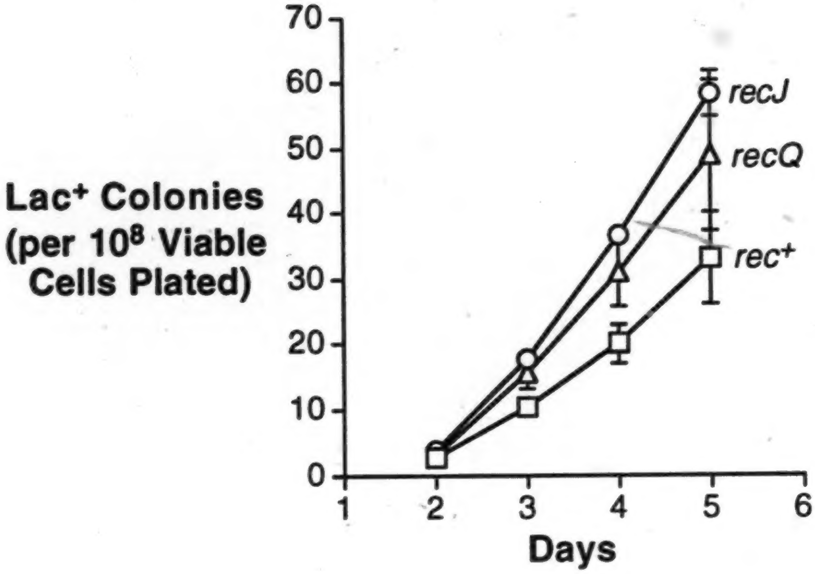


FIGURE 2-4. Genes required for alternative recombination pathways RecF and RecE are not required for adaptive mutation to Lac$^+$. Experiments are as in FIGURES 2-1 to 2-3. The *recJ284*::Tn*10* and *recQ61*::Tn*3* alleles were transduced into the *lacI-lacZ* frameshift strain (Cairns and Foster, 1991) by standard methods. For *rec*$^+$, n=44 except for day 5, on which n=22; for *recJ* and *recQ*, n=36 except for day 5, on which n=18. If it is significant, the slight increase in adaptive mutation in *recJ* and *recQ* strains could be explained as follows: Perhaps only RecBCD-mediated recombination can participate in the formation of adaptive mutations, and perhaps the debilitation of the RecF recombination pathway shunts more recombination into the RecBCD pathway. This would elevate mutation, just as a RecBCD pathway-specific hyper-rec mutation (*recD*) does.

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CHAPTER 3[*]

OPPOSING ROLES OF THE HOLLIDAY JUNCTION PROCESSING SYSTEMS OF *ESCHERICHIA COLI* IN RECOMBINATION-DEPENDENT ADAPTIVE MUTATION

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INTRODUCTION

Understanding the molecular mechanisms of spontaneous mutation is critical both to our understanding of the generation of genetic diversity that drives evolution, as well as the early events in cancer, in which mutagenesis underlies oncogenic transformation. For decades one mode of spontaneous mutation was believed to be exclusive. Spontaneous mutations were described as occurring before a cell experiences an environment in which the mutation might be useful, randomly in the genome, and were measured as mutations per cell per generation (Luria and Delbrück, 1943; Lederberg and Lederberg, 1952). The possibility of a fundamentally different mode of spontaneous mutation is emerging from studies of "adaptive" mutations in bacteria and yeast (see Ryan, 1955; 1959; Cairns *et al.*, 1988; Cairns and Foster, 1991; Hall, 1992; Jayaraman, 1992; Steele and Jinks-Robertson, 1992; reviewed by Foster, 1993). These occur only after exposure to a non-lethal genetic selection, in the apparent absence of cell division, and have been detected so far only in the genes whose functions were selected (references above but see Hall, 1990). These characteristics suggested that adaptive mutations might represent an example of Lamarckian evolution (Cairns *et al.*, 1988). Whether or not this is the case will be easier to discern once the molecular mechanisms of adaptive mutagenesis are understood.

It is already clear that there is more than one molecular mechanism by which adaptive mutations form (see Drake, 1991; Foster, 1993). Although little is known about the mechanism in most of the adaptive mutation assay systems, in one system significant molecular information exists. That system is reversion of a *lac* frameshift mutation carried on an F' episome in *Escherichia coli* cells (Cairns and Foster, 1991). In this system the following is known. First, the RecBCD system of homologous genetic recombination participates in adaptive but not growth-dependent Lac reversion (Harris *et al.*, 1994). Second, because RecBCD enzyme loads onto DNA only at double-strand breaks (DSBs) (Taylor, 1988), DSBs are implicated as a molecular intermediate in the adaptive mutagenesis (Harris *et al.*, 1994; see Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996). Third, the adaptive reversions of this +1 frameshift allele are nearly all -1 deletions in small mononucleotide repeats, whereas the growth-dependent Lac$^+$ reversions are highly heterogeneous (Foster and Trimarchi, 1994; Rosenberg *et al.*, 1994). Such simple repeat instability is characteristic of DNA polymerase error (Ripley, 1990) thought to be caused by a template slippage mechanism (Streisinger *et al.*, 1966). The adaptive reversion sequences resemble the simple repeat instability seen in hereditary non-polyposis colon cancer (reviewed by Modrich, 1994) and other cells that lack post-synthesis DNA mismatch repair (Levinson and Gutman, 1987; Cupples *et al.*, 1990; Strand *et al.*, 1993). Fourth, the hypothesis that the absence of functional mismatch repair is responsible for the unique sequence spectrum of the adaptive Lac$^+$ reversions is supported by the finding that mismatch repair-defective cells produce a growth-dependent Lac$^+$ reversion spectrum that is indistinguishable from that of the adaptive reversions (Longerich *et al.*, 1995).

These data have suggested a model (Harris *et al.*, 1994; see Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996) in which the stressed, starving cells generate DNA DSBs that promote RecBCD-mediated homologous recombination (see FIGURE 3-1-A). An invading 3' end in a RecA/RecBCD-promoted strand exchange intermediate was suggested to prime DNA synthesis during which polymerase errors are made. The errors might escape mismatch repair due to insufficient mismatch repair activity in these cells. The failure to detect mutations in unselected genes could be caused by DSB-mediated killing of cells that do not become Lac$^+$, and thus do not escape the starvation stress that promotes DSBs. [Note that F' episome loss causes death of the host cell (Jensen and Gerdes, 1995)]. Alternatively, perhaps unselected mutations would be found if other loci on the *lac*-bearing F' replicon were tested. This replicon might be particularly active in the recombination-dependent adaptive mutation mechanism.

A fifth piece of information from this system has provided a possible source of the DSBs, has encouraged the idea that the F' replicon is special, and is suggesting a different molecular mechanism: the F'-encoded proteins responsible for conjugative transfer of the plasmid (but not actual transfer) are necessary for high frequency Rec-dependent Lac$^+$ reversion, such that both transfer-defective F's and a chromosomal *lac* gene appear mutationally inactive (Foster and Trimarchi, 1995; Galitski and Roth, 1995; Radicella *et al.*, 1995). These authors hypothesize that conjugative transfer replication could be the source of the DNA polymerase errors that lead to adaptive mutation. Recombination is not usually required for transfer of conjugative plasmids or for transfer replication (Frost *et al.*, 1994) but could be necessary if the transfer replication were incomplete such that the newly synthesized fragment must be recombined into an intact replicon in order to preserve the mutation (FIGURE 3-1-B) (Foster and Trimarchi, 1995; Galitski and Roth, 1995; Peters and Benson, 1995). In this model, recombination acts after the polymerase error. A whole recombination reaction should be required to capture the fragment containing the error (see FIGURE 3-1-B). This would seem to be indistinguishable from bacterial conjugational recombination. This contrasts with the mechanism discussed previously (see FIGURE 3-1-A), in which the recombinational strand exchange intermediate itself primes the DNA synthesis during which the errors occur. If the strand exchange intermediates themselves are mutagenic (*i.e.* if only partial recombination reactions are necessary), then the recombination proteins required might differ from those for conjugational recombination whole reactions in that failure to resolve intermediates might promote mutation.

In this paper we investigate the role of recombination and recombinational strand exchange intermediates in adaptive Lac$^+$ reversion by manipulating the enzymes that process strand exchange intermediates into recombinant products. In *E. coli* conjugational recombination, strand exchange intermediates are resolved to products by either of two Holliday junction resolution systems (Lloyd, 1991; see West, 1994). We find that this is not the case for Lac$^+$ reversion. First, the two resolution systems, RecG and RuvABC, which appear redundant for conjugational recombination, affect Lac adaptive mutation in opposite ways, one inhibiting and the other promoting mutation. Second, delaying the action of both resolution systems causes hypermutation. These results imply that recombination intermediates themselves promote Lac$^+$ adaptive mutation.

MATERIALS AND METHODS

Bacterial Strains Strains used in this work are listed in TABLE 3-1 and APPENDIX I. All new genotypes were made by standard P1 transduction methods. Throughout the work, unless otherwise specified, the *ruvA* alleles used were *ruvA59*::Tn*10*, which is polar on *ruvB* creating RuvAB-deficiency, and a streptomycin-resistant derivative of this allele, *ruvA76*::Tn*10Sm*. *ruvA76*::Tn*10Sm* was constructed by disruption of the tetracycline resistance gene of Tn*10*, inserting the streptomycin-resistance cassette, using the method of François *et al.* (1987).

Mutation Assays Adaptive reversion assays were performed as described previously using the same media and conditions (Harris *et al.*, 1994), except that the *ruv recG* mutants were constructed and grown at ≤32°C to avoid accumulation of growth-defect-suppressing mutations and were assayed for Lac$^+$ reversion at 37°C. These procedures are as follows. Each strain to be assayed for adaptive mutation is taken directly from the original culture that was constructed and tested and frozen at -80°C. The strain is streaked for single colonies on M9 minimal medium containing vitamin B1 and 0.1% glycerol. Four to twelve independent cultures used in each experiment are inoculated, each from one whole (separate) single colony from the plate and grown to saturation in M9 B1 0.1% glycerol. The saturated cultures are washed twice in M9 B1 and resuspended to a concentration of viable cells that gives an assayable number of Lac$^+$ colonies over the duration of the experiment when 50-200 µl are mixed with an 8- to 40-fold excess of scavenger cells (grown up using the same procedure as just described) and plated in M9 B1 0.1% lactose top agar on M9 B1 0.1% lactose agar plates and incubated at 37°C. Two different dilutions of each separate culture are plated. These same saturated cultures are assayed for the number of viable cells on LB plates, and are tested for the presence of *rec* or *ruv* mutations. Because poorly viable genotypes such as all of the *ruv recG* combinations accumulate high frequencies of growth-defect-suppressor mutations and true reversion mutations (discussed below), we have found that it is imperative both to minimize growth of the cultures used in the experiments [*i.e.* avoid diluting and re-growing saturated cultures as in the procedure of Foster (1994)] and to test each of the actual cultures used in the adaptive reversion experiments for presence of *ruv* and *recG* alleles and for the absence of suppressor mutations (described below and further in the text).

Severe growth defects are caused by the double mutant combinations *ruvA recG*, *ruvB recG* and *ruvC recG*, such that cells carrying these combinations are genetically unstable--they readily accumulate growth-defect-suppressing mutations and also true reversions of the transposon-insertion null alleles (Lloyd, 1991; Mandal, 1993; R.S. Harris and S.M. Rosenberg, unpublished observations). Both the suppressor mutations and the true reversion mutations can be distinguished from their *ruv recG* parents by their larger colony size and by their increased UV-resistance (Lloyd, 1991; Mandal, 1993; R.S.

Harris and S.M. Rosenberg, unpublished observations). In addition to these phenotypes, we have found (reported below) that such suppressor and reversion strains behave differently in adaptive mutation experiments; they show severely decreased adaptive reversion whereas cultures of all of the *ruv recG* combinations that retain their extreme UV-sensitivity and small colony size display adaptive hypermutation (see RESULTS AND DISCUSSION for details). To ensure that the independent cultures used in adaptive reversion experiments are free from growth-defect-suppressor mutations and true reversions, the procedures were modified as follows. First, on the streak plate from which colonies for the saturated cultures used in the experiments are obtained, small colonies are chosen. Some large suppressor and revertant colonies are usually present but these are avoided. Second, the saturated cultures are grown slowly at 30-32°C rather than at 37°C. We have found that this reduces the frequency of large colony-forming, UV-resistant cells in the final cultures. Third, the saturated cultures used in the experiments are tested for UV-sensitivity, and for the presence of the transposon associated with the *ruv* and *recG* alleles as described above. Fourth, we showed that adaptive mutation selection conditions do not promote accumulation of suppressor or reversion mutations; the ratio of large to small colonies present in cultures plated is the same as that observed in *lac*$^-$ cells recovered after four days incubation under adaptive reversion conditions. Finally, for each *ruv* allele used in each *ruv recG* double mutant combination, two to three strains were constructed independently and shown to give the same results in adaptive mutation experiments when the precautions and testing described here were done for each. The occasional suppressor and reversion strains that we obtained have UV-resistance levels that range from comparable with a *ruv* single mutant (which is more resistant than any of the *ruv recG* double mutants) to as resistant as *rec*$^+$ cells. Those that we examined, at several different UV-resistance levels, showed adaptive hypomutation, in contrast with their *ruv recG* parents (RESULTS AND DISCUSSION). One of them, with a UV-resistance level comparable to a *ruv* single mutant, was in fact a revertant of *recG*, presumably by precise excision of the transposon disrupting that gene. We did not map or characterize further the other, growth-defect-suppressor mutant strains. Similar suppressor mutants characterized by Mandal *et al.* (1993) carry *rus* mutations, which activate an otherwise cryptic pathway of Holliday junction resolution.

The Lac$^+$ colonies arising over time are expressed per 10^8 viable cells plated (measured in the viable cell counts of the cultures to be plated). As previously, the number of viable frameshift-bearing cells was measured each day of the experiments and neither growth nor death was observed in any of the experiments reported here (see RESULTS AND DISCUSSION for data). Therefore in all experiments, with all strains used here, the number of viable cells plated is the number of viable cells that remained on the plates throughout the course of the experiments. Thus, the different mutation phenotypes reported represent mutations per viable cell on the plates.

Growth-dependent mutation rates were measured as described previously (Harris *et al.*, 1994). Mutation rates were calculated using the method of the median (Lea and Coulson, 1949) as modified by von Borstel (von Borstel, 1978).

RESULTS AND DISCUSSION

Experimental System The mutation assay system described by Cairns and Foster (Cairns and Foster, 1991) measures reversion of the *lacI33* +1 frameshift mutation carried on an F' episome in cells with the chromosomal *lac* operon deleted. The *lacI* and *lacZ* genes are fused such that the +1 frameshift mutation in *lacI* (*lacI33*) is polar on *lacZ* and the cells are Lac$^-$. Growth-dependent mutant colonies appear on the second day after plating on minimal lactose medium, and form independently of RecA and RecBCD proteins. Adaptive mutants arise continuously during the week after plating (Cairns and Foster, 1991) and do not arise in *recA*, or *recB* null mutant cells (Harris *et al*., 1994). The *lac*⁻ cells giving rise to the Lac⁺ mutants are prevented from multiplying on the minimal lactose medium by the presence of an excess of non-revertible, *lac*-deletion "scavenger" cells that consume any contaminating, non-lactose carbon sources that might be present. The absence of growth of the frameshift-bearing cells is confirmed by daily viable cell measurements in which a plug of agar from the plate is suspended in liquid and assayed for colony forming units on rifampicin plates which let the frameshift-bearing cell, but not the scavenger, form colonies. Such measurements showed no net growth or death for all of the experiments reported here.

The scavenger cell is also male, carrying an F' with no *lac* genes, in order to discourage transfer between the frameshift-bearing cell and the scavenger. In fact, about 8-10% of Lac⁺ adaptive revertants have transferred their F' into the scavenger (Radicella *et al*., 1995; Rosenberg *et al*., 1995). However, F' transfer appears to be unnecessary for, and probably occurs after, adaptive Lac reversion as shown by the following observations: first, mutations that decrease transfer by 10^5-fold decrease Lac⁺ adaptive reversion by only 10-fold (Foster and Trimarchi, 1995); and second, the RecA protein is required in the frameshift-bearing cell and not in the scavenger (Rosenberg *et al*., 1995 and data presented below). Thus, models in which transfer synthesis is thought to be critical for Lac⁺ adaptive reversion suppose that the transfer synthesis occurs without actual transfer (Foster and Trimarchi, 1995; Galitski and Roth, 1995).

The RuvABC and RecG Holliday Junction Resolution Systems Play Opposing Roles in Lac⁺ Adaptive Reversion Both the RecA and RecBCD proteins, which are necessary for Lac⁺ adaptive reversion, function early in recombination to initiate formation of strand exchange intermediates (reviewed by Rosenberg and Hastings, 1991;

West, 1992; Kowalczykowski, *et al.*, 1994). In conjugational recombination, the strand exchange intermediates are then resolved either by the RuvABC resolution system or by an alternative system that requires RecG (Lloyd, 1991; West, 1994). Thus, cells that carry *ruv* single mutations, or carry a *recG* mutation, are recombination-proficient. Only the *ruv recG* double mutant combinations produce recombination-deficiency (Lloyd, 1991).

To ask whether the genetic requirements for Lac⁺ adaptive reversion parallel those for conjugational recombination, we examined the effects of single mutations in the RecG and RuvABC systems on post-plating Lac⁺ reversion. In FIGURE 3-2-A, a *recG* null mutant shows greatly elevated post-plating Lac⁺ reversion. This contrasts with the phenotype of *recG* deficiency in conjugational recombination, in which a very small depression is seen (Lloyd, 1991). The hypermutation in a *recG* strain could be an elevation of genuine RecABC-dependent Lac⁺ adaptive reversion, but could also have been caused by the activation of some other, new, RecA-independent route to mutation. To distinguish these possibilities, a *recG recA* double mutant was tested. In FIGURE 3-2-B, the hypermutation in a *recG* strain is shown to be completely RecA-dependent. Thus, the presence of the RecG protein appears to antagonize RecA-dependent Lac⁺ adaptive reversion.

ruvA, *ruvB*, and *ruvC* mutations have little effect on conjugational recombination in the presence of a functional *recG⁺* gene (see Lloyd, 1991). This is not the case for Lac⁺ adaptive reversion. In FIGURE 3-2-C, the data show that post-plating Lac⁺ reversion is strongly inhibited in *ruvA* and *ruvB* null mutants and is abolished in a *ruvC* null mutant strain. Two different *ruvA* alleles show the same effect: *ruvA200*, which blocks only RuvA function, and *ruvA59*::Tn*10*, which is also polar on the *ruvB* gene. We conclude that the RuvABC system is necessary for Lac⁺ adaptive reversion even in the presence of functional RecG. This is unlike normal conjugational recombination. A possible similarity between Lac⁺ adaptive reversion and two unusual recombination assay systems that show *ruv* dependence in the presence of RecG (Lloyd, 1991; Matic *et al.*, 1995) is discussed below.

A possible biochemical basis for the opposite effects of the two resolution systems on Lac⁺ adaptive reversion is considered below. For now we wish to conclude, first, that the genetic requirements of Lac⁺ adaptive reversion and conjugational recombination are different. This discourages fragment capture models for Lac⁺ adaptive reversion. Second, the enzymes in these resolution systems are well defined biochemically: RuvAB and RecG proteins bind to, and perform branch migration of, Holliday junctions and other strand exchange intermediates; RuvC endonuclease binds to and then cleaves such intermediates, assisted by RuvAB (West, 1994). The involvement of all of these proteins provides evidence that strand exchange intermediates are also intermediates in Lac⁺ adaptive reversion. The data in TABLE 3-2 show that growth-dependent, RecA-independent Lac⁺ reversion rates are unaffected by these proteins.

Temporary Absence of Both the RuvABC and RecG Resolution Systems Promotes Lac⁺ Adaptive Hypermutation Conjugational recombination is blocked by the absence of RuvA, RuvB, or RuvC, and RecG proteins simultaneously, presumably because both routes to resolution of strand exchange intermediates are blocked (Lloyd, 1991). Under this situation, the strand exchange intermediates should accumulate but should not produce recombinant products. If strand exchange intermediates themselves were responsible for priming the DNA synthesis that leads to recombination-dependent Lac⁺ adaptive mutation (Harris *et al.*, 1994; FIGURE 3-1-A), then it is possible that blocking both resolution pathways in *ruv recG* double mutants might cause an increase in Lac⁺ adaptive reversion. This is seen for a *ruvC recG* double mutant and a *ruvA recG* double mutant in FIGURE 3-3, A and B, respectively. The *ruvA* allele is polar on *ruvB*.

We also observe this effect with a different *ruvA* allele, *ruvA200*, a non-polar allele, in combination with *recG*, and with the *ruvB recG* double mutant combination [data not shown; *ruvA200 recG* and *ruvB recG* are SMR1563 and SMR1565, APPENDIX I]. The hypermutation in *ruvA recG* and *ruvC recG* is completely RecA-dependent (*e.g.* see FIGURE 3-3-B) and thus represents enhancement of normal recombination-dependent Lac$^+$ adaptive reversion, not creation of a novel mutagenic route. The *ruv recG* resolution-defective mutation combinations do not affect growth-dependent Lac$^+$ reversion rates (TABLE 3-2).

All of the *ruv recG* double mutant combinations used here have impaired growth compared with *rec*$^+$ and with *ruv* and *recG* single mutant cells, and all of them readily accumulate growth defect-suppressing mutations and true reversion mutations (Lloyd, 1991; Mandal, 1993; R.S. Harris and S.M. Rosenberg, unpublished observations). The suppressor-containing strains and revertants are distinguishable from true *ruv recG* strains by their increased colony size and increased UV-resistance. Special precautions were taken here to avoid accumulation of such mutants and to verify that every culture used in adaptive mutation experiments was free from such mutations (see MATERIALS AND METHODS). Our procedure uses cultures derived each from a single (small) colony and grown to saturation (MATERIALS AND METHODS). The procedure of Foster (1994), which involves growth of a saturated culture, dilution and regrowth to saturation, resulted in cultures with increased UV-resistance. These behaved differently in adaptive reversion experiments, showing very low levels of reversion comparable to those seen for *ruv* single mutant strains (data not shown). Although one culture that we tested was a *recG* true revertant (*i.e.* a *ruv* single mutant), this phenotype of depressed mutation was also seen for cultures carrying growth defect-suppressor mutations, as evidenced by their UV-resistance level which was higher than *ruv* single mutants. Mandal *et al.* (1993)

characterized suppressor mutants arising in *ruv recG* strains as mutations in *rus* which activate an otherwise cryptic Holliday junction resolution system.

We have found that *ruvA recG*, *ruvB recG*, and *ruvC recG* strains manifest *recA*-dependent adaptive hypermutation. An obvious conclusion is that, again, Lac$^+$ adaptive reversion has different genetic requirements from conjugational recombination. Fragment capture models for the mutagenesis are not supported, and in this case it appears that the idea that intermediates themselves are mutagenic is supported by these data. The data imply that accumulation of strand exchange intermediates in the doubly resolvase-defective cells causes increased RecA-dependent Lac$^+$ reversion.

A somewhat less obvious consideration is that, taken at face value, these data would seem to imply that it is possible to recover viable mutant colonies without ever resolving the strand exchange intermediates that promoted the mutations. This perplexing possibility will be disputed by the data to follow, which, in summary, will indicate that resolution is actually required but that when cells are *ruvA* or *ruvB* or *ruvC* and *recG* defective, the resolution occurs after the intermediate is transferred into the *rec*$^+$ scavenger cells. Although transfer of recombination intermediates was not expected by us, the following lines of evidence lead us to suggest this possibility.

First, we noted that the magnitude of the hypermutation effect caused by *ruv recG* double mutations is variable from experiment to experiment. This can be seen, for example, by comparing the different magnitudes of the *ruvC recG* and *ruvA recG* hypermutation effects relative to *rec*$^+$ in FIGURE 3-3, A and B (and also varies between experiments with a single strain). For this reason a quantitative comparison of hypermutation between *recG* and *ruv recG* strains has not been done. We have not observed such variability with any other *rec* or single *ruv* mutations tested here or previously (Harris *et al*., 1994; Rosenberg *et al*., 1995). We have determined that this variability is caused by small variations in the proportion of the *ruv recG* frameshift-bearing cells relative to the *rec*$^+$ scavenger cells. When varied systematically, we find that decreasing the number of *rec*$^+$ scavenger cells relative to *ruv recG* frameshift-bearing cells greatly increases the amount of adaptive Lac$^+$ reversion caused by *ruv recG*. In FIGURE 3-4, experiments conducted in parallel using 1×10^8 frameshift-bearing cells mixed with either 8×10^8 or 4×10^9 scavenger cells show high and normal levels of adaptive mutation, respectively. These data are highly repeatable and the elevated mutation is not caused by growth of the frameshift-bearing cells in the presence of fewer scavengers (FIGURE 3-4, B and D; also FIGURE 3-3-C). These data suggest the following hypothesis: perhaps strand exchange intermediates must eventually be resolved in order to recover viable cells and perhaps this resolution occurs after transfer of the F', with its unresolved recombination intermediate, into a *rec*$^+$ scavenger cell. If the persistence of the unresolved strand exchange intermediate is mutagenic, then a delay in finding a *rec*$^+$ scavenger cell with which to mate would increase mutation. Thus, we hypothesize that the fewer scavenger cells plated, the longer the *ruv recG* frameshift cell waits to transfer into a *rec*$^+$ scavenger cell, and the more mutations are promoted, though, ultimately, the intermediates promoting them must be resolved.

The idea that the *ruv recG* hypermutation events must resolve eventually in the *rec*$^+$ scavenger cell is supported by the following observations. First, we find that nearly all of the Lac$^+$ revertants isolated from *ruv recG* experiments contain the rifampicin-sensitivity marker and wild-type *ruv* and *recG* genes which are present on the scavenger cell chromosome but not on the chromosomes of the frameshift-bearing strains (Cairns and Foster, 1991). The numbers of isolated Lac$^+$ revertants carrying the scavenger cell's chromosomal markers were 15/15 (*ruvC recG*), 13/15 (*ruvA59 recG*), 16/16 (*ruvA200 recG*), and 14/16 (*ruvB recG*). This is not the case for *recG* and *rec*$^+$ frameshift-bearing cells, which produced only 3 out of 14 (*recG*, this work), and 9 out of 116 [*rec*$^+$, (Rosenberg *et al.*, 1995)] Lac$^+$ adaptive revertants carrying the chromosomal rifampicin-sensitivity marker from the scavenger cell. Therefore, in the *ruv recG* experiments, most surviving Lac$^+$ revertants transferred into the scavenger cell.

Second, when *ruvA recG*, *ruvB recG*, or *ruvC recG* frameshift-bearing cells are plated with scavenger cells that are either *ruvC recG* (FIGURE 3-5-A) or *ruvC* (FIGURE 3-5-B), Lac$^+$ adaptive reversion is abolished. This demonstrates a requirement for RuvC-dependent resolution functions in the scavenger cell when the frameshift-bearing cell is unable to resolve recombination intermediates, *i.e.*, is *ruv recG*. The scavenger cell genotype is irrelevant to mutation levels observed in *rec*$^+$, *recA*, *ruvA*, *ruvB*, *ruvC*, or *recG* cells (Rosenberg *et al*., 1995; data in FIGURE 5; and data not shown). We favor the hypothesis that it is resolution that must occur in the scavenger, rather than an entire, normal conjugational recombination reaction, occurring perhaps after a single strand is transferred, for two reasons. First, a *ruvC* single mutation in the scavenger also abolishes Lac$^+$ adaptive reversion with *ruv recG* frameshift-bearing cells (FIGURE 3-5-B). This is unlike conjugational recombination, in which *recG*$^+$ substitutes for *ruvC*$^+$ (Lloyd, 1991), and is like the requirement for *ruv* genes seen in FIGURE 3-2-C. Second, there is no requirement for RecA protein in the scavenger cell (FIGURE 3-5-C). Thus, it appears that it is not necessary to initiate strand exchange in the scavenger, but merely to resolve an already-formed intermediate.

An alternative explanation might be that the *ruv recG* scavengers are simply poor recipients of conjugation and thus do not admit the transferred F'. Lloyd (Lloyd, 1991) observed a 10-fold decrease in the ability to act as a transfer recipient in a *ruvC recG* strain. However, this explanation cannot explain the requirement for *ruvC*$^+$ function in the scavenger cells (FIGURE 3-5-B). *ruvC* cells are reasonably proficient recipients of transfer, showing only a 2-fold decrease with respect to *rec*$^+$ (Lloyd, 1991), but yet a *ruvC* mutation in the scavenger cell completely blocks adaptive reversion of *ruv recG* strains plated with it. Thus the idea that the resolution functions of the RuvABC system are required in the scavenger is supported.

The results presented above imply that when Holliday junction resolution is completely blocked the accumulation of strand exchange intermediates is hypermutagenic. This supports the idea that strand exchange intermediates prime DNA synthesis during which polymerase errors occur. Second, the data imply that although mutagenic, strand exchange intermediates must be resolved to recover viable mutant colonies, and that this resolution occurs after transfer into the scavenger cell.

FURTHER DISCUSSION

Conclusions from the data reported here can be summarized as follows. First, the presence of RecG protein inhibits Lac$^+$ adaptive reversion. Second, the RuvABC proteins are required for Lac$^+$ adaptive reversion. Involvement of these junction-specific proteins implies that strand exchange intermediates are also intermediates in Lac$^+$ adaptive reversion. Third, accumulation of strand exchange intermediates in doubly resolvase-defective cells is hypermutagenic, but requires eventual exposure to the RuvABC proteins, presumably for eventual resolution. Apparently, that resolution can occur after transfer of the presumed unresolved intermediate into a scavenger cell. It seems as though delaying that transfer by reducing the number of scavengers increases Lac$^+$ adaptive reversion. These results support models in which the recombinational strand exchange intermediate is mutagenic. One such model envisions that a 3' end which has invaded a homologous duplex directly primes the DNA synthesis in which polymerase errors occur and that these become the mutations (FIGURE 3-1-A). Other models are possible.

The idea that strand exchange intermediates might be transferred into another cell was very surprising to us in view of conventional assumptions that only single-strand DNA is transferred (see Frost *et al.*, 1994). However, L. Frost (Edmonton) made us aware that the idea is not without precedent. Wong and Paranchych (1976) found evidence for transfer of RNA molecules containing secondary structures through pili.

The results of Foster *et al.* (1996) were kindly shared with us prior to publication, during the preparation of this manuscript. They obtain results similar to ours for *ruv* and *recG* singly mutant strains. However with *ruvA recG*, *ruvB recG* and *ruvC recG* double mutants they report depressed adaptive reversion which they argue is not the result of growth-defect-suppressor or reversion mutations. We report adaptive hypermutation of such double mutants, which is demonstrated not to result from growth-defect-suppressor or reversion mutations, and which depends on successful transfer of the F' into a *ruv*$^+$ scavenger cell. It is possible that the absence of hypermutation in their experiments may be caused by experimental conditions that are not favorable for transfer. Ultimately, both labs find that the recombination intermediates must be resolved for recovery of viable Lac$^+$ revertants. Because our conditions allow recovery of transferred molecules, we were able to observe the hypermutation that appears to result when resolution is delayed until transfer into a *ruv*$^+$ scavenger cell.

Opposing Roles of the RuvABC and RecG Systems A possible explanation for why RecG protein inhibits Lac$^+$ adaptive reversion, whereas the RuvABC system promotes it, is suggested by the biochemistry of these proteins. Each of these resolution systems consists of a branch-migration component plus a resolution component. The branch-migration components are an association of RuvA and RuvB proteins (RuvAB) for the RuvABC system, and the RecG protein for the RecG system (see West, 1994). Branch-migration must precede resolution. The RuvABC system's resolvase is RuvC, and the resolvase for the RecG system has not yet been identified. Biochemically, the branch-migration proteins are helicases (see West, 1994; Whitby *et al.*, 1994) and, like many DNA helicases, they have preferred strand polarities. For the RuvAB and RecG branch migration helicases, their polarities are detectable on RecA-coated DNA substrates, and on such substrates the two have opposite strand polarities (Whitby *et al.*, 1993; Whitby and Lloyd, 1995). We will suggest a model that uses the opposite strand polarities of the RuvAB and RecG branch migration components in order to explain the following facts: first, RecG inhibits, whereas RuvABC facilitates Lac$^+$ adaptive reversion; second, the functions of these systems are redundant and necessary for conjugational recombination.

The model is presented in FIGURE 3-6. First, in RecABCD-mediated recombination, there is evidence that both 5' and 3' single-strand DNA ends may form RecA-promoted strand exchange intermediates with a homologous duplex (Dutreix *et al.*, 1991; Rosenberg and Hastings, 1991; Rinken *et al.*, 1992; Miesel and Roth, 1996; Razavy *et al.*, 1996). Both intermediates are presumed to lead to recombination products (top right and bottom left of FIGURE 3-6), but only the 3' end invasions are suggested to lead to adaptive mutation, because only the 3' ends can prime the DNA synthesis during which polymerase errors occur (FIGURE 3-6, lower left). We suggest that a 3' end invasion intermediate is extended by RuvABC, but is unwound and undone by RecG (FIGURE 3-6, left); and that the converse happens to a 5' end invasion intermediate (FIGURE 3-6, right), which is extended and resolved by the RecG system but is undone by RuvAB. That is, resolution of the intermediates of each polarity is proposed to be specific to the resolvase system. This can explain why these systems have opposite effects on Lac$^+$ reversion (proposed to be active with 3' ends only) and redundant effects on conjugational recombination (occurs well enough with either intermediate). See FIGURE 3-6 for further discussion and an alternative view of these enzymes.

Two cases of recombination are known in which the RuvABC system is necessary in the presence of functional RecG: recombination of ColE1-based plasmids (Lloyd, 1991) and conjugational recombination between the 85% identical (homeologous) DNAs of *E. coli* and *Salmonella* (Matic *et al.*, 1995). We suggest that in both cases, only 3' end invasions will work. For plasmid recombination, this could be because it is RecBCD-independent and uses components of another *E. coli* recombination pathway, RecF (Luisa-Deluca *et al.*, 1989). The RecF pathway may use 3' invasions exclusively because of its use of a 5' exonuclease, RecJ. In the case of homeologous recombination, perhaps only 3' ends work because DNA synthesis primed at the joint is a necessity for achieving a long, stable heteroduplex junction with no DNA mispairs in it. The mispairs, we suggest, destabilize the junctions because of the many proteins that interact with such DNA distortions (see also Priebe *et al.*, 1994).

How Much Mutation Results from Blocking Ruv and RecG Resolution Routes? Previous measurements of the number of transfers occurring between frameshift-bearing cells and scavengers estimate that only 8-10% of Lac$^+$ revertants had transferred into the scavenger (Radicella *et al.*, 1995; Rosenberg *et al.*, 1995). It seems reasonable that the same percent of transfers may occur in *ruvC recG* cells. If so, the hypermutation events scored in our experiments with *ruv recG* cells plated with *rec*$^+$ scavenger cells may be only 10% of the hypermutation that occurred. The rest of the mutational events would be lost because they are not transferred and not resolved into viable molecules.

Molecular Mechanism of Lac$^+$ Adaptive Mutation Our results support models in which strand exchange intermediates somehow promote mutation, and almost certainly, more models are possible than have been considered here. One version of our suggestion that strand exchange intermediates prime the DNA synthesis that leads to the mutation was considered by Kuzminov (1995; see also Foster *et al.*, 1996). In this version branch migration is used to migrate the newly synthesized, error-containing DNA into a region where its complementary strand is also new and thus unmethylated. This would prevent mismatch repair from correcting the error properly because its strand discrimination would be lost. This version is inconsistent with our unpublished and Foster *et al.*'s (1995) observation that overexpression of mismatch repair proteins decreases adaptive reversion. This could occur only if the polymerase errors were correctable, *i.e.* present in hemimethylated DNA.

Why are the conjugational transfer proteins required for Lac$^+$ adaptive reversion, whereas transfer itself is not (Foster and Trimarchi, 1995; Galitski and Roth, 1995; Radicella *et al.*, 1995)? Perhaps their action at the origin of transfer, *oriT*, on the F' leads to the required DSB (Rosenberg *et al.*, 1995; 1996). The transfer proteins make a single-strand nick at *oriT* (see Frost *et al.*, 1994) which could lead to a DSB by any of several mechanisms. If the sole function of the transfer proteins is generation of single-strand nicks that lead to DSBs that serve as RecBCD loading sites, then we expect that there will be chromosomal locations that can utilize the Rec-dependent mutation mechanism being uncovered in this system. Although much of the bacterial chromosome is cold for RecBCD-promoted recombination, and so presumably has few DSBs, there are sites that are hot (Louarn *et al.*, 1991; Asai *et al.*, 1993) and these may be mutationally active.

Tests of the hypotheses presented here will be revealing. In this system, and for others in which recombination is implicated in formation of mutations (Demerec, 1962; Magni and von Borstel, 1962; Paszewski and Surzycki, 1964; Esposito and Bruschi, 1993; Strathern *et al.*, 1995), further work on the molecular mechanisms will be informative.

TABLE 3-1. ***E. coli*** **K-12 strains.**

Strain[a]	Relevant genotype	Reference[a]
Frameshift-bearing cells		
FC40	*ara* Δ(*lac-proAB*)$_{\text{XIII}}$ *thi* Rif^r [F′ *proAB*$^+$ *lacI33* Ω*lacZ*]	[b]
SMR624	FC40 Δ(*srlR-recA*)*306*::Tn*10*	[c]
RSH38	FC40 *ruvC53 eda51*::Tn*10*	This work
RSH45	FC40 *ruvC53 eda51*::Tn*10 recG258*::Tn*10miniKan*	This work
RSH152	FC40 *ruvA200 eda51*::Tn*10*	This work
RSH154	FC40 *ruvA59*::Tn*10*	This work
RSH155	FC40 *ruvB9 zea3*::Tn*10*	This work
RSH159	FC40 *ruvA200 eda51*::Tn*10 recG258*::Tn*10miniKan*	This work
RSH160	FC40 *ruvA59*::Tn*10 recG258*::Tn*10miniKan*	This work
RSH161	FC40 *ruvB9 zea3*::Tn*10 recG258*::Tn*10miniKan*	This work
RSH275	FC40 *ruvA76*::Tn*10Sm* Δ(*srlR-recA*)*306*::Tn*10* *recG258*::Tn*10miniKan*	This work
RSH316	FC40 *recG258*::Tn*10miniKan*	This work
RSH326	FC40 *recG258*::Tn*10miniKan* Δ(*srlR-recA*)*306*::Tn*10*	This work
Scavenger cells		
FC29	*ara* Δ(*lac-proAB*)$_{\text{XIII}}$ *thi* Rif^r [F′ *proAB*$^+$ Δ*lac*]	[b]
RSH9	FC29 Δ(*srlR-recA*)*306*::Tn*10*	[d]
RSH353	FC29 *ruvC53 eda51*::Tn*10*	This work
RSH355	FC29 *ruvC53 eda51*::Tn*10 recG258*::Tn*10miniKan*	This work

[a] Construction details are in APPENDIX I.

[b] Cairns and Foster, 1991.

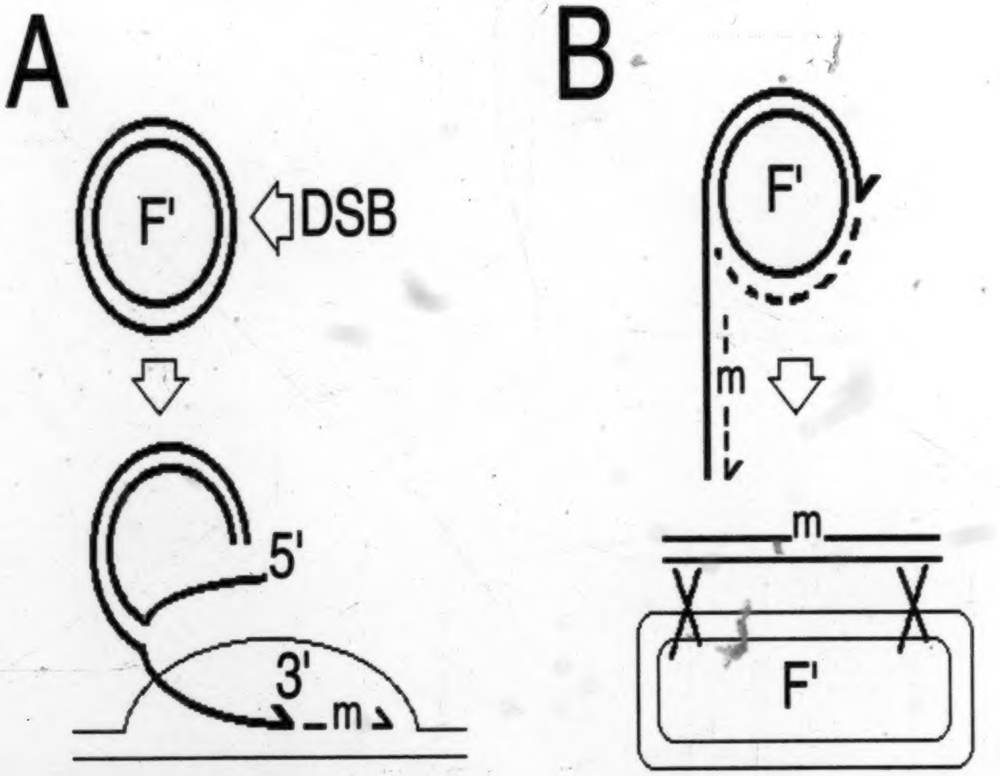
[c] Harris *et al.*, 1994.

[d] Rosenberg *et al.*, 1995.

TABLE 3-2. Mutation rates in growing cultures. Strains *rec*$^+$, *recG*, *ruvA*, *ruvC*, *ruvA recG*, *ruvC recG* and *recG ΔrecA* are FC40, RSH316, RSH154, RSH38, RSH160, RSH45, and RSH326, respectively (TABLE 3-1). Mutation rates are calculated by the method of the median (Lea and Coulson, 1949, as modified by von Borstel, 1978) and are measured as determined previously (Harris *et al.*, 1994). The *recG* strain displays extreme Lac$^+$ adaptive hypermutation (FIGURE 3-2) and also appears hypermutable in growth-dependent Lac$^+$ reversion here. The apparent elevation of growth-dependent mutation might be due to contamination of the preplating revertants with postplating, RecA-dependent adaptive revertants. This possibility is supported by the finding that the increase in *recG* is entirely *recA*$^+$-dependent (experiments 6 and 7). The RecA-independent, growth-dependent Lac$^+$ reversion rate is unaffected by *recG*.

Genotype	Experiment	Number of cultures	Rate of mutation to Lac$^+$ (mutations per cell per generation)
rec$^+$	1	40	4.7×10^{-10}
	2	40	7.2×10^{-10}
	3	40	9.6×10^{-10}
	4	40	6.4×10^{-10}
	5	40	7.5×10^{-10}
recG	3	40	20×10^{-10}
	4	40	15×10^{-10}
	5	40	22×10^{-10}
ruvA	1	39	4.5×10^{-10}
	2	40	5.7×10^{-10}
	5	40	4.0×10^{-10}
ruvC	1	40	4.0×10^{-10}
	2	40	3.3×10^{-10}
	5	40	2.6×10^{-10}
ruvA recG	1	26	3.6×10^{-10}
	2	34	8.0×10^{-10}
	5	40	16×10^{-10}
ruvC recG	1	33	9.4×10^{-10}
	2	31	28×10^{-10}
	5	40	5.5×10^{-10}
rec$^+$	6	10	4.7×10^{-10}
	7	10	4.0×10^{-10}
recG	6	10	29×10^{-10}
	7	9	61×10^{-10}
recG ΔrecA	6	10	3.0×10^{-10}
	7	4	1.7×10^{-10}

FIGURE 3-1. Two models for recombination-dependent mutations using polymerase errors. **A.** From Harris *et al.* (1994). The DSB is proposed to occur at the origin of transfer as a consequence of single-strand nicking by the transfer proteins (discussed in the text). **B.** From Peters and Benson (1995); Galitski and Roth (1995); Foster and Trimarchi (1995). Dashed lines represent newly synthesized DNA; "m", a polymerase mistake that becomes a mutation. DSB, DNA double-strand break. Large X's in B signify crossover recombination whole reactions. Models containing aspects of both models shown here are also possible. In both models, a DNA homology with the F' is required for recombination, and in both, that homology is imagined to be a sister replication product. Sister molecules might be infrequent in starving cells. However, the occurrence of adaptive revertants is also infrequent and so is not discouraged by this concern.


A
F'
DSB
5'
3'
m
B
F'
m
m
F'

recombination

primes synthesis

fragment capture

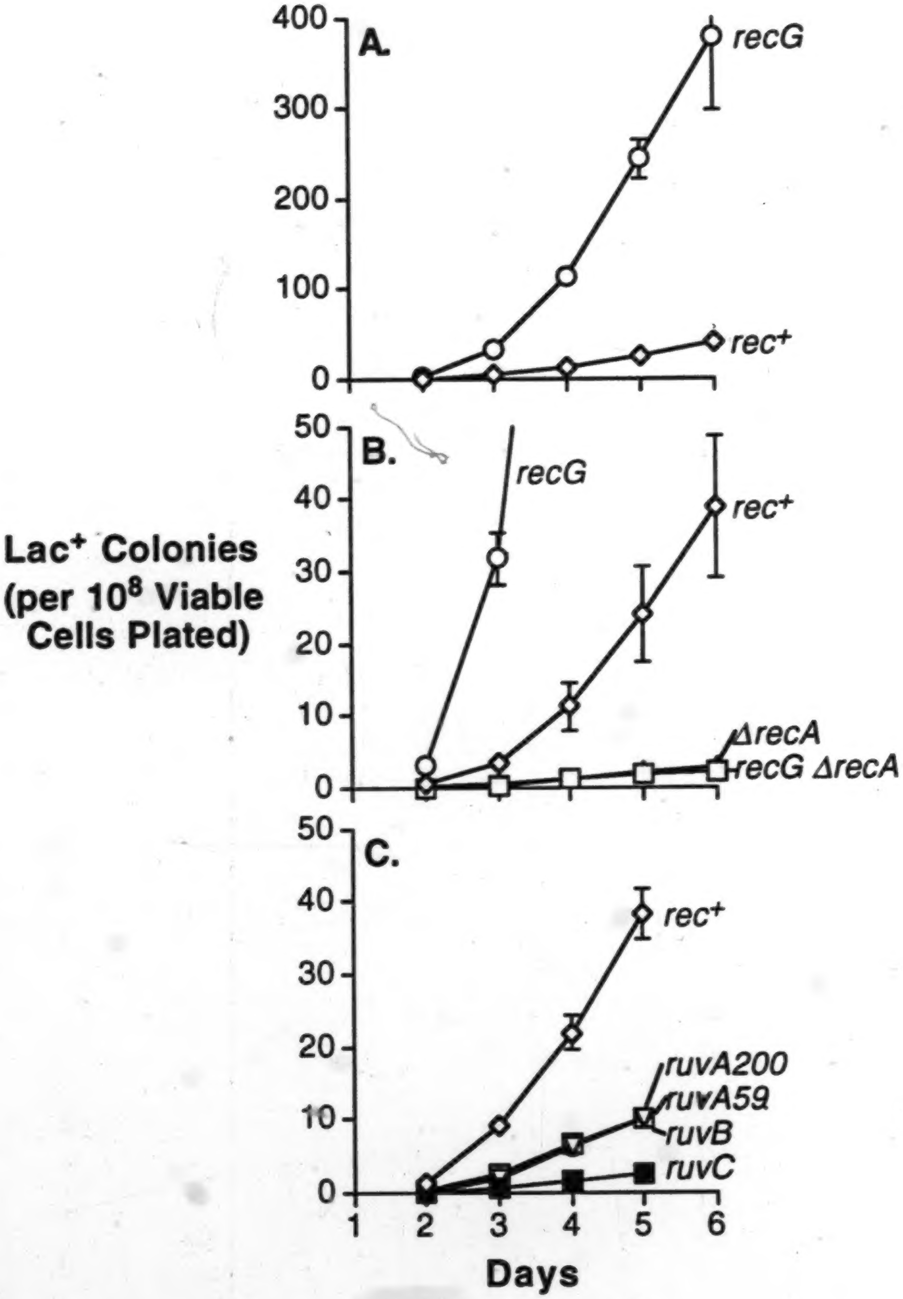
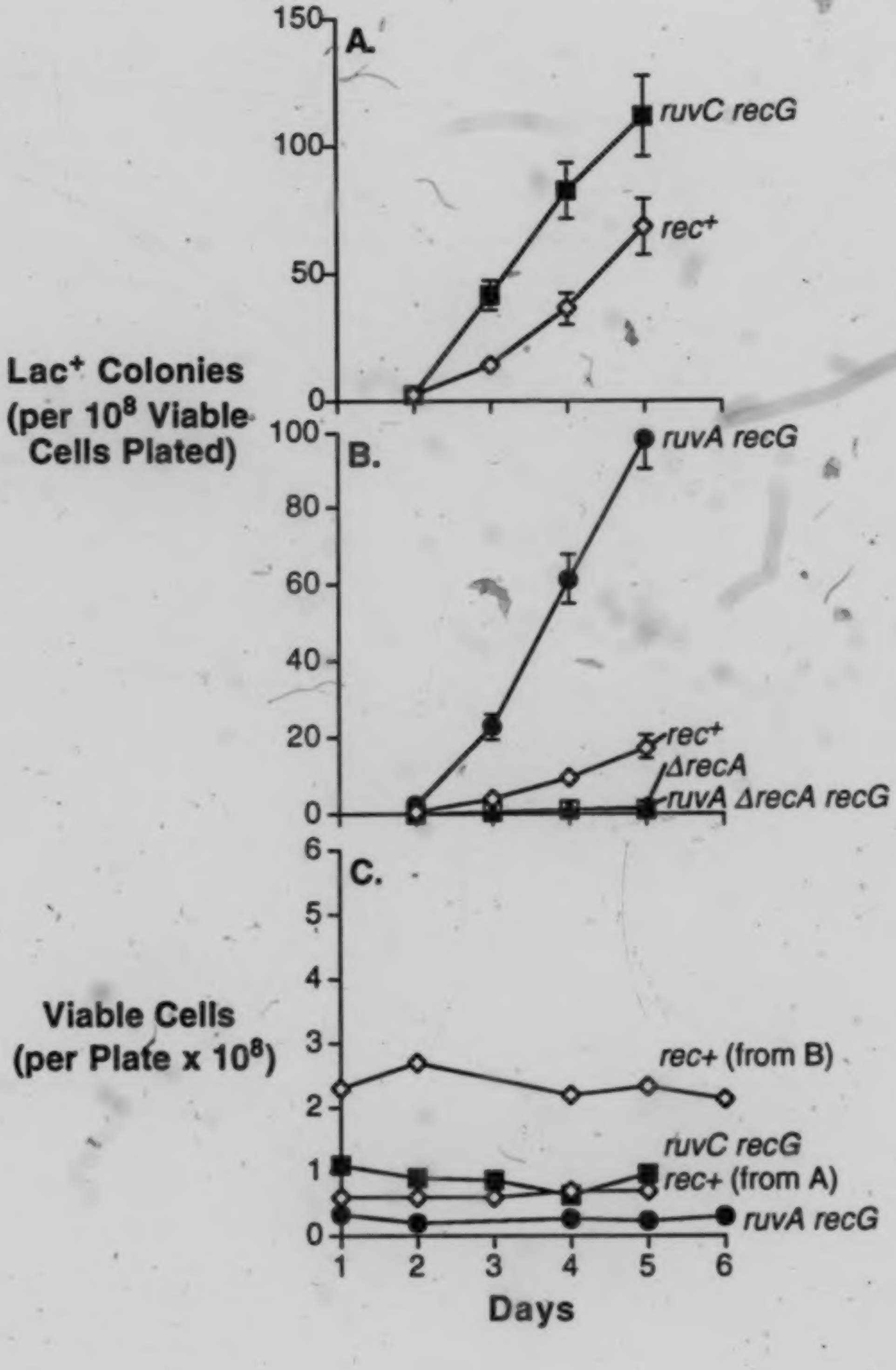


FIGURE 3-2. Opposing roles of the RuvABC and RecG resolution systems in Lac$^+$ adaptive reversion. Error bars represent one standard error of the mean.

FIGURE 3-3. Lac⁺ adaptive hypermutation in cells defective for both RuvABC and RecG resolution systems. A. Hypermutation in a *ruvC recG* strain. B. Hypermutation in a *ruvA recG* strain. Strains RSH160 and RSH275 (TABLE 3-1) carry *ruvA* polar mutations that also create *ruvB*-deficiency (MATERIALS AND METHODS). C. Viable cell measurements of the *lac⁻* rifampicin-resistant frameshift-bearing cells during the experiments displayed in A. and B. Error bars represent one standard error of the mean.



A.
B.
C.
Lac⁺ Colonies (per 10⁸ Viable Cells Plated)
Viable Cells (per Plate x 10⁸)
150
100
50
0
80
60
40
20
6
5
4
3
2
1
ruvC recG
rec⁺
ruvA recG
rec⁺
ΔrecA
ruvA ΔrecA recG
rec+ (from B)
ruvC recG
rec+ (from A)
ruvA recG
1
2
3
4
5
6
Days

FIGURE 3-4. Hypermutation of *ruv recG* double mutants varies inversely with the number of *rec*⁺ scavenger cells. **A.** and **C.** Open symbols represent an experiment with a 40:1 ratio of *rec*⁺ scavenger cells to *ruv recG* frameshift-bearing cells (4 × 10⁹ scavengers + 1 × 10⁸ frameshift-bearers); closed symbols indicate an 8:1 ratio (8 × 10⁸ scavengers + 1 × 10⁸ frameshift-bearers). **B.** and **D.** Viable cell measurements of the *lac*⁻ rifampicin-resistant frameshift-bearing cells during the experiments displayed in A and C, respectively. These show that decreasing the number of scavengers does not promote hypermutation by allowing growth of the frameshift-bearing cell. Under extremely hypermutagenic conditions (filled symbols), the frameshift-bearing cells do not multiply. Error bars represent one standard error of the mean.

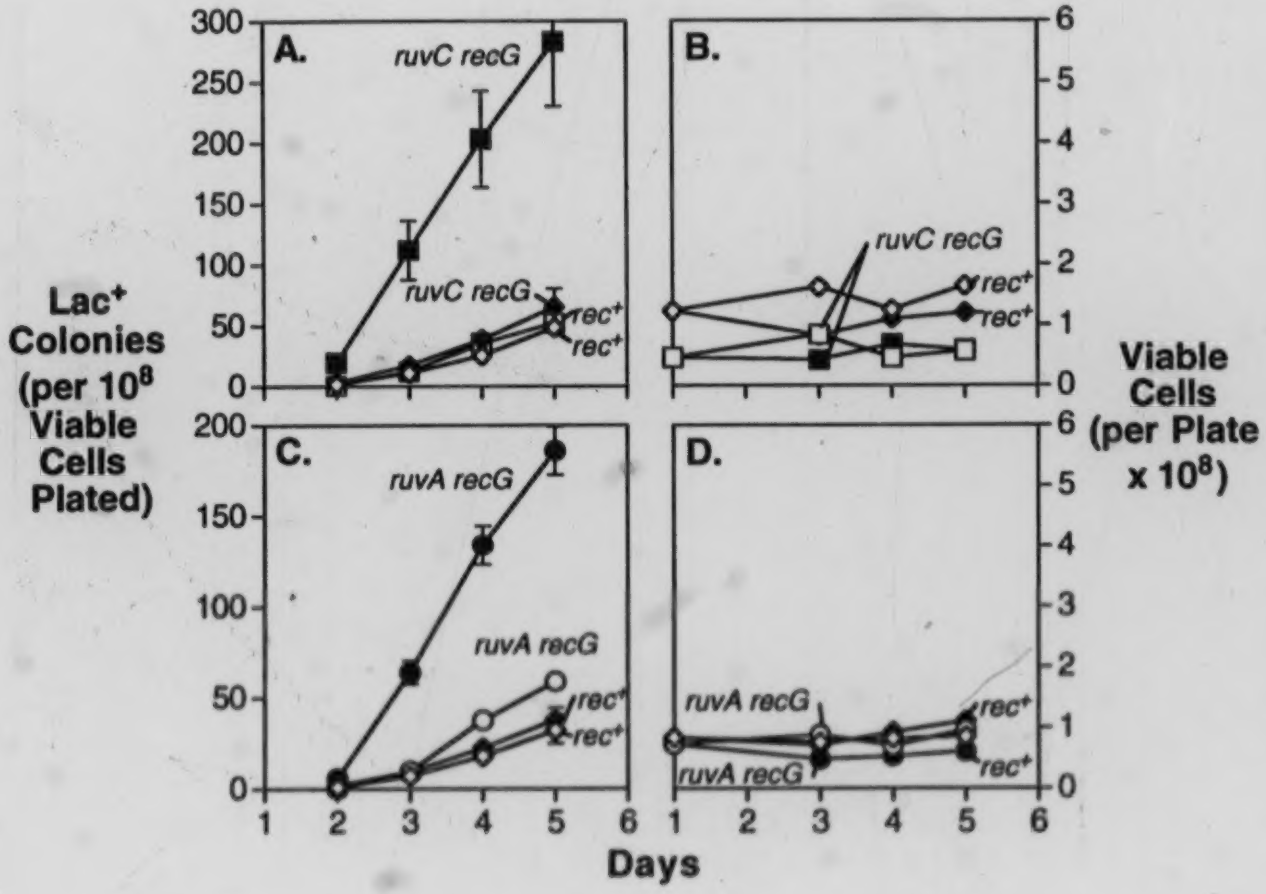

A.
ruvC recG
ruvC recG
rec+
rec+
B.
ruvC recG
rec+
rec+
C.
ruvA recG
ruvA recG
rec+
rec+
D.
ruvA recG
ruvA recG
rec+
rec+
300
250
200
150
100
50
0
200
150
100
50
0
6
5
4
3
2
1
0
1
2
3
4
5
6
Lac+ Colonies (per 10^8 Viable Cells Plated)
Viable Cells (per Plate x 10^8)
Days

FIGURE 3-5. Hypermutation of *ruv recG* double mutants requires functional resolution proteins in the scavenger cells. Discussed in the text. **A.** Adaptive hypermutation of *ruvA recG*, *ruvB recG*, and *ruvC recG* cells is abolished by plating with a *ruvC recG* scavenger cell, and, **B.** by plating with a *ruvC* scavenger cell. This indicates that functional resolution proteins of the RuvABC system are needed in the scavenger cell for recovery of the Lac$^+$ adaptive mutants from these doubly resolvase-defective strains. **C.** Functional *recA* is not required in the scavenger cell for recovery of adaptive mutants from a *ruvA recG* strain. Error bars represent one standard error of the mean.

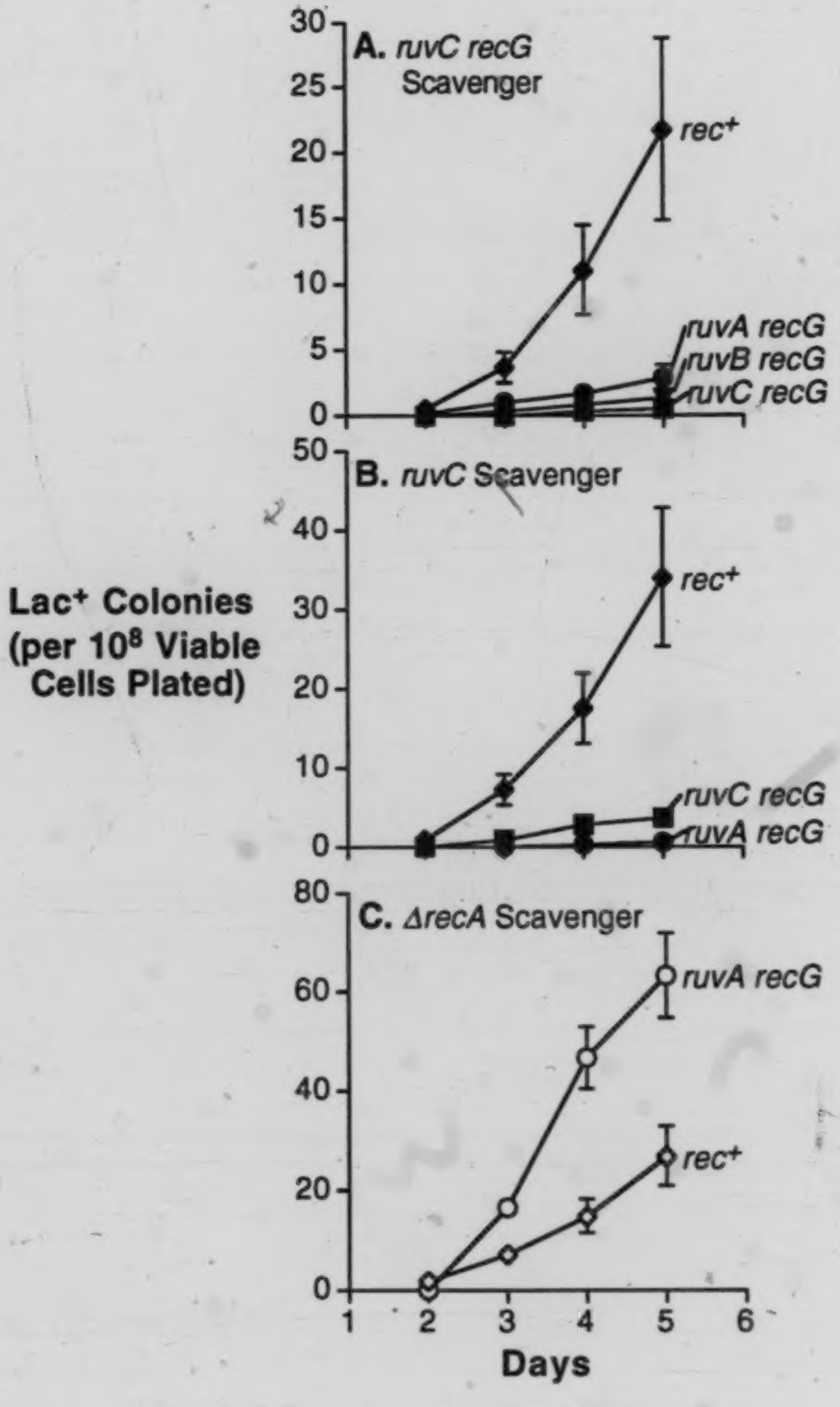
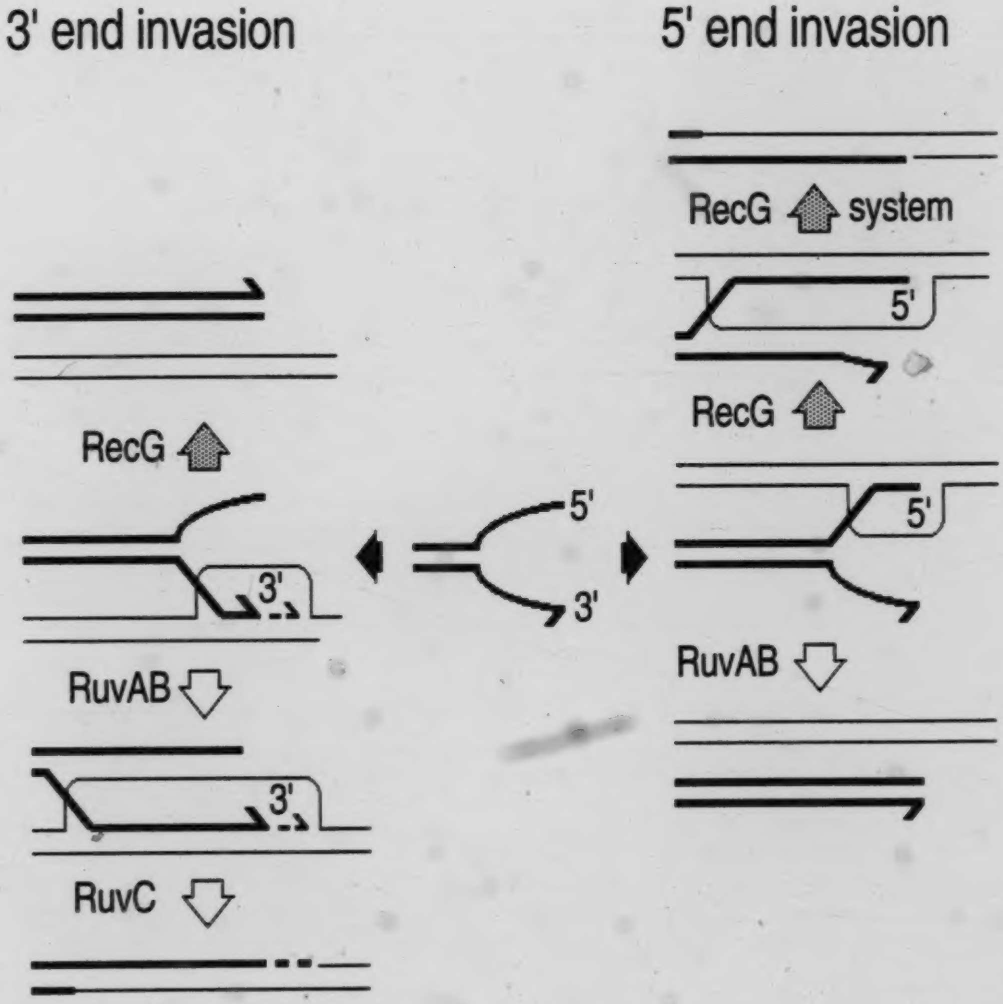
Lac+ Colonies (per 10^8 Viable Cells Plated)
A. ruvC recG Scavenger
rec+
ruvA recG
ruvB recG
ruvC recG
0
5
10
15
20
25
30
B. ruvC Scavenger
rec+
ruvC recG
ruvA recG
0
10
20
30
40
50
C. ΔrecA Scavenger
ruvA recG
rec+
0
20
40
60
80
1
2
3
4
5
6
Days

FIGURE 3-6. Opposite polarities of the RuvAB and RecG branch migration components can explain opposing roles of the two resolution systems in Lac$^+$ adaptive mutation. Parallel lines represent strands of DNA. Dashed lines indicate newly synthesized DNA. The central molecule is unwound by RecBC protein (not drawn) and the strand invasions to the left and right of it are catalyzed by RecA (not drawn) (Rosenberg and Hastings, 1991). The model suggests that each resolution system treats the two invasion intermediates in opposite ways, such that the RuvABC system resolves 3' end invasions but undoes 5' end invasions, whereas the RecG system resolves 5' end invasions but undoes 3' end invasions. "RecG system" is indicated at the resolution step in the upper right to denote a Holliday junction resolvase that works with RecG helicase. The resolvase of the RecG system has not yet been identified (see West, 1994). Both systems function in conjugational recombination (Lloyd, 1991), in which both 3' and 5' ends are proposed to contribute to products (Rosenberg and Hastings, 1991). In adaptive mutation, only the 3' ends are proposed to be active because only these can prime DNA synthesis. Thus, RecG is inhibitory and RuvABC is necessary for Lac reversion. The specific polarities suggested are opposite those proposed by Whitby and Lloyd (1995). Because circular single-strand DNAs, not linear molecules, were used in their assay, it seems possible that our model is not inconsistent with their data; circular and linear DNAs have given apparently different polarities in strand exchange assays before (Konforti and Davis, 1987). For clarity, only one of the possible recombination products is shown.


3' end invasion
5' end invasion
RecG system
5'
RecG
RecG
3'
5'
3'
5'
RuvAB
RuvAB
3'
RuvC

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CHAPTER 4*

A DIRECT ROLE FOR DNA POLYMERASE III IN ADAPTIVE REVERSION OF A FRAMESHIFT MUTATION IN *ESCHERICHIA COLI*

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[*] A version of this chapter is in press: Harris, R.S., H.J. Bull, and S.M. Rosenberg (1997) *Mutation Research*, **375**.

INTRODUCTION

Unlike normal, spontaneous growth-dependent mutations, adaptive mutations arise in non-dividing or slowly-growing cells, only after exposure to selective conditions, and have been found only in genes whose functions were selected (Cairns *et al.*, 1988; Hall, 1990; Cairns and Foster, 1991; Hall, 1992; Steele and Jinks-Robertson, 1992; Foster, 1993). In one experimental system, the adaptive mutations also occur *via* a fundamentally different molecular mechanism (Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996). Reversions of a +1 frameshift mutation in a *lacI*::*lacZ* fusion gene in *Escherichia coli* (Cairns and Foster, 1991) uniquely require recombination proteins of the RecBCD pathway (Harris *et al.*, 1994; Foster *et al.*, 1996; Harris *et al.*, 1996) and display a different mutation spectrum than growth-dependent Lac reversions (Foster and Trimarchi, 1994; Rosenberg *et al.*, 1994).

The adaptive Lac$^+$ reversions are almost all single base deletions in mononucleotide repeats. Such simple repeat instability suggests DNA polymerase errors that have escaped correction by post-synthesis mismatch repair (Levinson and Gutman, 1987; Cupples *et al.*, 1990; Strand *et al.*, 1993; Modrich, 1994). Cells undergoing Lac$^+$ adaptive mutation appear to experience a transient deficiency in mismatch repair: First, inactivation of mismatch repair produces a growth-dependent mutation spectrum indistinguishable from that of adaptive mutations (Longerich *et al.*, 1995). Second, mismatch repair protein levels (Feng *et al.*, 1996) and function (Harris *et al.*, 1997) decrease in stationary-phase, starving cells undergoing adaptive Lac reversion. Thus, adaptive Lac$^+$ reversions appear to result from DNA polymerase errors, perhaps caused by a template slippage mechanism (Streisinger *et al.*, 1966; Ripley, 1990), which persist due to inadequate mismatch repair capacity. We will address here which DNA polymerase is responsible.

The major replicative polymerase of *E. coli*, DNA polymerase III (PolIII), was implicated as the primary source of the adaptive mutations (Foster *et al.*, 1995). An antimutator allele of *dnaE* (*dnaE915*), which encodes the main subunit of the PolIII holoenzyme, caused a roughly 3-fold decrease in adaptive Lac reversion (Foster *et al.*, 1995; see also FIGURE 4-1 and TABLE 4-2 of this study). This could imply that PolIII makes adaptive reversions, however, an antimutator PolIII could also affect mutation indirectly through modulation of the MutHLSU system. During rapid cell growth, errors made by a proofreading-defective PolIII saturate the mismatch repair system (Damagnez *et al.*, 1989; Schaaper and Radman, 1989). During starvation, mismatch repair proteins are also limiting (Harris *et al.*, 1997). Therefore, the antimutator PolIII could act indirectly by releasing more mismatch repair activity which would then correct errors made by other DNA polymerase(s). To ascertain which DNA polymerase makes adaptive mutations these alternate hypotheses must be distinguished.

We report here that the antimutator PolIII acts independently of mismatch repair level, supporting a direct role for DNA PolIII in recombination-dependent adaptive mutation.

MATERIALS AND METHODS

Bacterial Strains Strains used are listed in TABLE 4-1 (see also APPENDIX I, TABLE I-1). *lac* frameshift-bearing strains are isogenic derivatives of FC40 (Cairns and Foster, 1991) constructed by standard P1 transduction methods.

Mutation Assays Adaptive reversion assays were performed as described previously (Harris *et al.*, 1994; 1996). For comparison in TABLE 4-2, growth-dependent and adaptive mutants were scored as the number of Lac⁺ colonies that appeared on day 2 + 3 and day 4 + 5 of the experiment, respectively. Viable cell counts (Harris *et al.*, 1994; 1996) show that neither growth nor death of the *lac⁻* frameshift-bearing cells occurred over the course of any of the experiments reported here (*e.g.* FIGURE 4-3-B).

RESULTS AND DISCUSSION

We asked whether *dnaE915* can still lower recombination (Rec)-dependent adaptive Lac reversion in *mutL* and *mutS* null mutant strains, a result that would be possible only if the *dnaE915* effect were independent of mismatch repair levels. This experiment is complicated by the fact that *mutL* and *mutS* mutations cause severe hypermutation in the adaptive reversion assay (Foster and Cairns, 1992; FIGURE 4-2; FIGURE 4-3; TABLE 4-2). Thus, it is critical to distinguish whether the hypermutation that occurs in *mutL* and *mutS* strains is *bona fide* Rec-dependent Lac reversion (Harris *et al.*, 1994), or whether the hypermutation represents activation of some other Rec-independent mutagenic route. We find that the hypermutation in *mutL* and *mutS* strains is *recA*-dependent (FIGURE 4-2). This clarifies interpretation of our results (below) and of others' (Foster and Cairns, 1992) by showing that the same Rec-dependent mutation pathway operates in *mutL* and *mutS* as in *mut⁺* cells.

We find that the *dnaE915* antimutator allele decreases Lac⁺ adaptive reversion in *mutL* cells (FIGURE 4-3; TABLE 4-2) and in *mutS* cells (TABLE 4-2). The depressions of observed Lac⁺ colonies on day 4 and day 5 caused by *dnaE915* in *mutL* and *mutS* cells, relative to *dnaE⁺* isogenic strains, indicate that *dnaE915* acts independently of mismatch repair. These data exclude the possibility that *dnaE915* lessens adaptive mutation by sparing mismatch repair activity to correct errors made by another polymerase, and support a direct role for PolIII in Rec-dependent adaptive mutation.

dnaE915 also decreases growth-dependent mutation to Lac+ in this assay system (see Lac+ colonies, days 2 and 3, TABLE 4-2; also Foster *et al.*, 1995). Although this appears to contradict the finding of Schaaper (1993) that *dnaE915* increases the frequency of forward frameshift mutations in *lacI*, examination of those data reveals that only frameshift mutations at A:T base pairs are increased significantly in *dnaE915* cells whereas those at C:G base pairs are not (Table 3 of Schaaper, 1993). For Lac reversion in our assay system, 19/25 growth-dependent -1 frameshift mutations are deletions of a C:G base pair (Table 2 of Foster and Trimarchi, 1994; Table 1 of Rosenberg *et al.*, 1994). Thus, the decrease of growth-dependent reversion to Lac+ caused by *dnaE915* could be due to fewer deletions of C:G base pairs.

The involvement of proteins necessary for homologous recombination in adaptive mutation led us to suggest the possibility that recombinational strand-exchange intermediates prime DNA synthesis during which errors occur, and that these errors become adaptive mutations (Harris *et al.*, 1994). Evidence that strand-exchange intermediates promote adaptive mutation supports this view (Harris *et al.*, 1996). Because no *E. coli* DNA polymerase has been shown to be specific for DNA synthesis associated with recombination, any of the three known DNA polymerases were plausible candidates. The data reported here, and those of Foster *et al.* (1995), imply that PolIII performs DNA synthesis that can result in Rec-dependent adaptive mutations. An understanding of the connections between recombination, DNA synthesis and mutation will be facilitated by these results.

TABLE 4-1. *Escherichia coli* K-12 strains used in this study.

Strain	Relevant genotype	Reference or construction
Frameshift-bearing cells:		
FC40	*ara* Δ(*lac-proB*)$_{\mathrm{XIII}}$ *thi* Rif^{r} [F′ *proAB*$^{+}$ *lacI33*::*lacZ*]	Cairns and Foster, 1991
RSH334	FC40 *zae*::Tn*10d*-Cam *dnaE915 zae-502*::Tn*10*	P1 NR9915 x FC40
RSH335	FC40 *zae*::Tn*10d*-Cam *dnaE*$^{+}$ *zae-502*::Tn*10*	P1 NR9918 x FC40
RSH356	FC40 *zae*::Tn*10d*-Cam *dnaE915 zae-502*::Tn*10 mutL211*::Tn*5*	P1 SMR620 x RSH334
RSH358	FC40 *zae*::Tn*10d*-Cam *dnaE*$^{+}$ *zae-502*::Tn*10 mutL211*::Tn*5*	P1 SMR620 x RSH335
RSH360	FC40 *zae*::Tn*10d*-Cam *dnaE915 zae-502*::Tn*10 mutS201*::Tn*5*	P1 SMR438 x RSH334
RSH361	FC40 *zae*::Tn*10d*-Cam *dnaE*$^{+}$ *zae-502*::Tn*10 mutS201*::Tn*5*	P1 SMR438 x RSH335
RSH587	FC40 *mutS201*::Tn*5*	P1 SMR438 x FC40
RSH591	FC40 *mutS201*::Tn*5* Δ(*srlR-recA*)*306*::Tn*10*	P1 SMR624 x RSH587
RSH593	FC40 *mutL211*::Tn*5*	P1 SMR620 x FC40
RSH594	FC40 *mutL211*::Tn*5* Δ(*srlR-recA*)*306*::Tn*10*	P1 SMR624 x RSH593

TABLE 4-1 contd.:

Scavenger cells:		
FC29	*ara* Δ*(lac-proB)*$_{\text{XIII}}$ *thi* [F' *proAB*$^{+}$ Δ*(lacI-lacZ)*]	Cairns and Foster, 1991
P1 donors used in strain constructions:		
NR9915	*zae*::Tn*10d*-Cam *dnaE915 zae-502*::Tn*10*	Fijalkowska *et al.*, 1993
NR9918	*zae*::Tn*10d*-Cam *dnaE*$^{+}$ *zae-502*::Tn*10*	Fijalkowska *et al.*, 1993
SMR438	*mutS201*::Tn*5*	Rosenberg lab collection
SMR620	*mutL211*::Tn*5*	Longerich *et al.*, 1995
SMR624	Δ*(srlR-recA)306*::Tn*10*	Harris *et al.*, 1994

TABLE 4-2. Effect of the *dnaE915* antimutator mutation on adaptive mutation in mismatch repair-deficient cells.

Relevant genotype [a]	Observed Lac$^+$ colonies per 10^8 cells plated (mean ± SEM)			Decrease in day 4 + 5 Lac$^+$ colonies in *dnaE915* relative to *dnaE*$^+$ cells
	Experiment	Day 2 + day 3	Day 4 + day 5 [b]	
dnaE915 mut$^+$	1	3 ± 1	10 ± 2	3
	2	5 ± 1	13 ± 1	4
dnaE$^+$ *mut*$^+$	1	8 ± 1	31 ± 3	
	2	14 ± 3	56 ± 11	
dnaE915 mutL	2	430 ± 64	110 ± 15	23
	3	340 ± 67	86 ± 13	58
dnaE$^+$ *mutL*	2	420 ± 30	2500 ± 230	
	3	990 ± 94	5000 ± 570	
dnaE915 mutS	2	770 ± 60	130 ± 10	4

TABLE 4-2 contd.:

	3	1000 ± 85	200 ± 19	4
dnaE⁺ mutS	2	540 ± 37	520 ± 33	
	3	750 ± 71	830 ± 61	

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[a] Strains *dnaE915* , *dnaE⁺*, *dnaE915 mutL*, *dnaE⁺ mutL*, *dnaE915 mutS* and *dnaE⁺ mutS* are RSH334, RSH335, RSH356, RSH358, RSH360 and RSH361 respectively (TABLE 4-1).

[b] *mutL* and *mutS* affect the appearance of day 4 and day 5 Lac⁺ colonies differently. The reason for this difference is not clear.

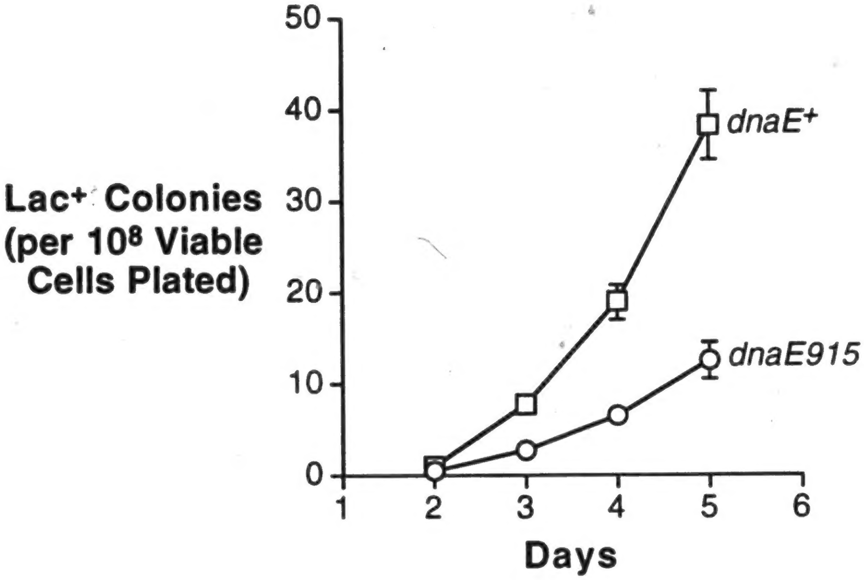

FIGURE 4-1. An antimutator mutation in *dnaE*, *dnaE915*, decreases Lac$^+$ adaptive reversion. See also Foster *et al.* (1995). Strains *dnaE915* and *dnaE*$^+$ are strains RSH334 and RSH335, respectively (TABLE 4-1). Error bars, one standard error of the mean.

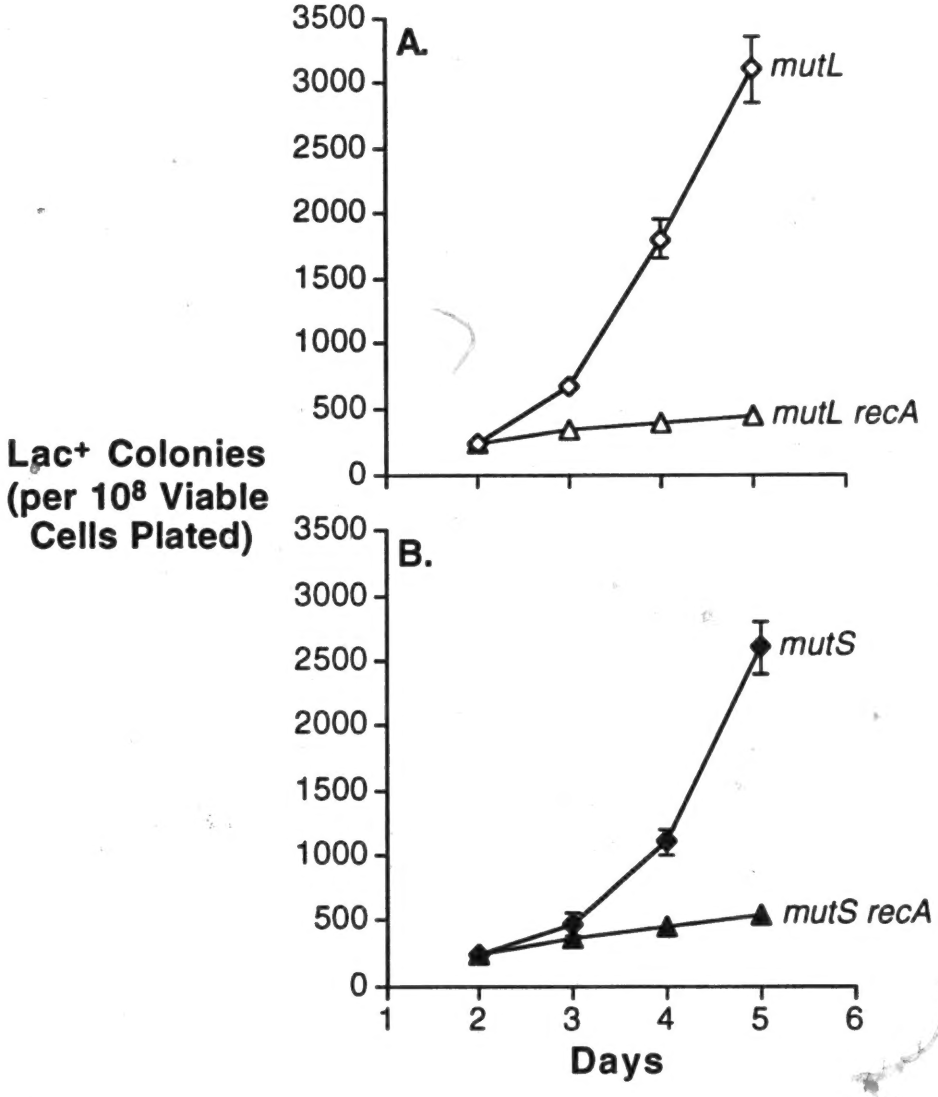

FIGURE 4-2. Adaptive hypermutation in *mutL* (A) and in *mutS* (B) strains is *recA*-dependent. Strains *mutL*, *mutL recA*, *mutS* and *mutS recA* are strains RSH593, RSH594, RSH587 and RSH591, respectively (TABLE 4-1). Error bars, one standard error of the mean.

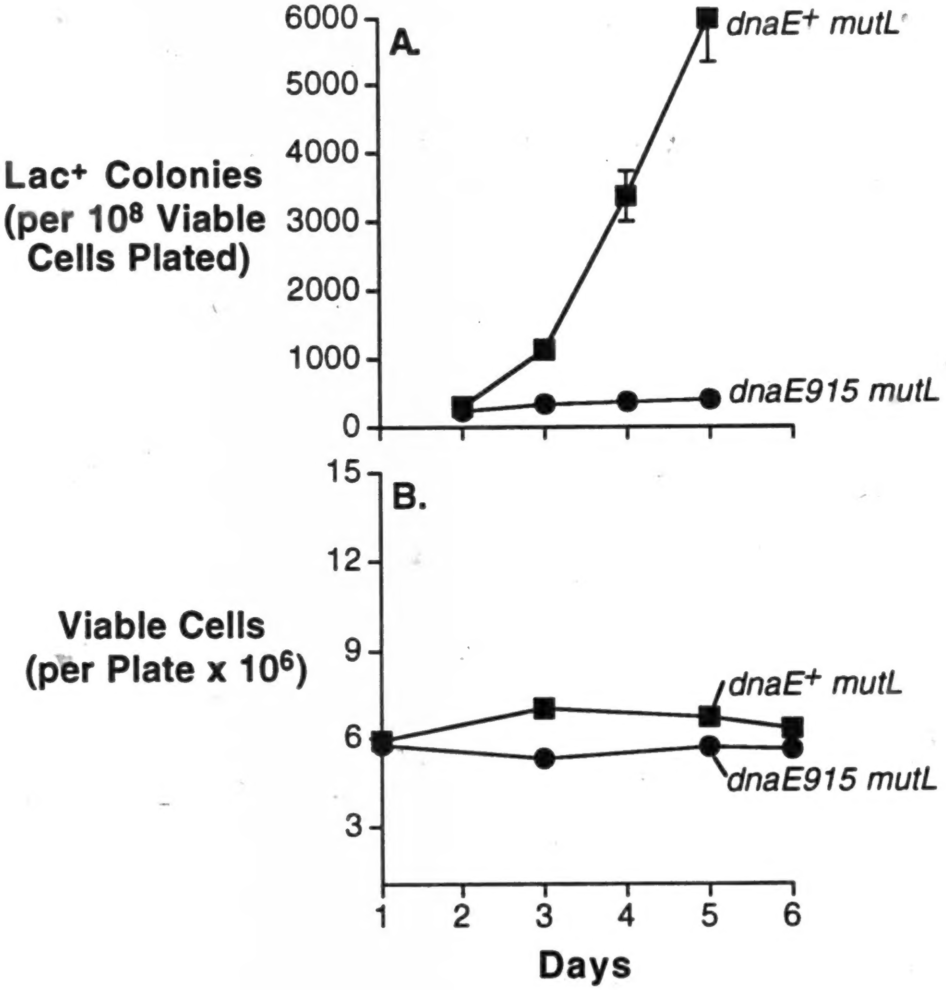

FIGURE 4-3. A. *dnaE915* decreases adaptive mutation independently of mismatch repair function. These data are an alternate representation of those found in TABLE 4-2, EXPERIMENT 2. **B. Neither growth nor death of the frameshift-bearing cell population was detected over the course of the experiments.** Thus, the decrease in mutations in *dnaE915 mutL* relative to *dnaE*+ *mutL* can not be attributed to death of the former or to growth of the latter strain. Strains *dnaE915 mutL* and *dnaE*+ *mutL* are strains RSH356 and RSH358, respectively (TABLE 4-1). Error bars, one standard error of the mean.

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CHAPTER 5*

LAC+ ADAPTIVE MUTANTS ARE NOT HERITABLY MUTATOR

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[*] The data reported here were discussed but not shown by Longerich, S., A.M. Galloway, R.S. Harris, C. Wong, and S.M. Rosenberg (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 12017-12020.

INTRODUCTION

An *E. coli* strain that yields mutants more frequently than an isogenic derivative, is said to display a mutator phenotype. A 10- to 1000-fold increase in mutation frequency (mutants per cell plated) is a strong mutator phenotype and is observed in cells lacking the methyl-directed mismatch repair system (Modrich, 1991). To determine whether Lac$^+$ adaptive mutation is correlated with a heritable inactivation of mismatch repair, the mutator phenotype of 20 independent Lac$^+$ adaptive mutants was assessed. The results presented here show that 20 out of 20 Lac$^+$ adaptive mutants are not heritably mutator.

MATERIALS AND METHODS

E. coli strains used here are listed in TABLE 5-1 and in greater detail in APPENDIX I. LBH broth (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 2µg/ml thymine, pH 7) + 0.1% glucose was used for all overnight cultures. All incubations were at 37°C for 24 hours. Mutator phenotypes were assayed as follows: (i) For mutation to nalidixic acid resistance (Nal^r), 100µl of a saturated overnight cultures were spread onto LBH plates (as broth, but solidified with 1.5% agar) and a small amount of nalidixic acid powder was dotted onto each plate. Nal^r colonies in the zone of clearing were scored after incubation. Alternatively, 10µl of a saturated overnight cultures were spotted onto LBH plates containing 4µg/ml nalidixic acid. The numbers of Nal^r colonies in the spot were scored. (ii) For mutation to streptomycin- or spectinomycin-resistance (Str^r, Spc^r), 10µl of saturated overnight cultures were spotted onto LBH plates containing 100µg/ml str or 200µg/ml spc, and the number of Str^r and Spc^r colonies was scored.

RESULTS AND CONCLUSIONS

TABLE 5-1 summarizes the results of the qualitative assessment of the mutator phenotype of Lac$^+$ adaptive mutants. Five mismatch repair-defective mutants used as positive controls for mutator phenotype (Modrich, 1991), displayed greater numbers of Nal^r, Str^r, and Spc^r colonies than the Lac$^+$ adaptive mutants. The *mut*$^+$ parent of all strains tested, FC40 (Cairns and Foster, 1991) which was never exposed to the lactose selection, showed a nonmutator phenotype similar to the Lac$^+$ adaptive mutants. The data show that 20 out of 20 adaptive Lac$^+$ mutants are not heritably mutator. Thus, the apparent absence of mismatch repair function during recombination-dependent adaptive mutation (Longerich *et al.*, 1995) must be transient.

TABLE 5-1. Observed numbers of Nal^r, Str^r, and Spc^r colonies.

Strain number [a]	Relevant characteristics	Number of mutant colonies (Nal^r) [b]	(Nal^r) [c]	Str^r	Spc^r
Nonmutator strain:					
FC40	*mut*$^+$ Lac$^-$	0	40	0	0
Mutator strains:					
SMR620	*mutL* Lac$^-$	28	TMTC [d]	11	6
SMR621	*mutU* Lac$^-$	9	TMTC	2	2
SMR622	*mutS* Lac$^-$	21	TMTC	3	2
SMR623	*mutH* Lac$^-$	26	TMTC	15	4
SMR843	*dam* Lac$^-$	5	TMTC	3	1
Lac$^+$ adaptive mutants:					
SMR1167	Lac$^+$	0	50 [e]	0	0
SMR1171	Lac$^+$	0	50 [e]	0	0
SMR1172	Lac$^+$	0	50 [e]	0	0
SMR1176	Lac$^+$	0	50 [e]	0	0
SMR1179	Lac$^+$	0	50 [e]	0	0
SMR1182	Lac$^+$	1	50 [e]	0	0
SMR1186	Lac$^+$	0	50 [e]	0	0
SMR1188	Lac$^+$	0	50 [e]	0	0
SMR1189	Lac$^+$	1	50 [e]	0	0
SMR1190	Lac$^+$	0	50 [e]	0	0
SMR1231	*recD* Lac$^+$	0	19	0	0
SMR1233	*recD* Lac$^+$	0	13	0	0
SMR1235	*recD* Lac$^+$	0	16	0	0
SMR1239	*recD* Lac$^+$	0	20 [e]	0	0
SMR1240	*recD* Lac$^+$	0	20 [e]	0	0
SMR1244	*recD* Lac$^+$	0	15 [e]	0	0
SMR1245	*recD* Lac$^+$	0	50 [e]	0	0
SMR1246	*recD* Lac$^+$	0	50 [e]	0	0
SMR1249	*recD* Lac$^+$	0	20 [e]	0	0
SMR1251	*recD* Lac$^+$	0	20 [e]	0	0

[a] Strain numbers are lab designations (see APPENDIX I).

[b] Determined from the zone of clearing method (MATERIALS AND METHODS).

[c] Determined from the spot method (MATERIALS AND METHODS).

[d] TMTC, too many colonies to count (*i.e.* ≥ 500 colonies/plate).

[e] Estimation based on FC40 which displayed 40 Nal^r colonies.

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CHAPTER 6*

MISMATCH REPAIR PROTEIN MUTL BECOMES LIMITING DURING STATIONARY-PHASE MUTATION

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In *Escherichia coli* mismatch repair is the single largest contributor to avoidance of mutations due to DNA polymerase errors in replication (Radman, 1988; Modrich, 1991). Mismatch repair also promotes genetic stability by editing the fidelity of genetic recombination, transposon excision, and by the involvement of its component proteins in transcription-coupled DNA repair and very-short-patch repair (Radman, 1988; Modrich, 1991; Lieb and Shehnaz, 1995; Mellon and Champe, 1996). The mismatch repair proteins are highly conserved throughout evolution and appear to play similar roles in simple and complex eukaryotes as they do in bacteria (Reenan and Kolodner, 1992; Modrich, 1994; de Wind *et al.*, 1995; Baker *et al.*, 1995; Baker *et al.*, 1996; Datta *et al.*, 1996; Hunter *et al.*, 1996; Kolodner, 1996). These proteins act on incorrectly paired and unpaired bases in DNA that arise *via* DNA synthesis errors, recombination of diverged sequences, and DNA damage. In all of these circumstances, mismatch repair enforces genetic stability. The consequences of failing to maintain this enforcement are profound for speciation (Rayssiguier *et al.*, 1989; Radman and Wagner, 1993; Matic *et al.*, 1995; Hunter *et al.*, 1996; Zahrt and Maloy, 1997), and for formation of cancers (Modrich, 1994; 1995; Kolodner, 1996).

Although central to maintenance of genetic stability, little is known about whether the mismatch repair system might be regulated (but see Stahl, 1988; Rayssiguier *et al.*, 1989; Hastings and Rosenberg, 1992; Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996; Longerich *et al.*, 1995 for hypotheses). If mismatch repair were regulated, then cells would regulate their potential to evolve. Two lines of work from *E. coli* have converged on the first evidence in any organism suggesting that mismatch repair proteins might be regulated, and more specifically, have suggested the down-regulation of mismatch repair during the differentiated states of stationary phase and nutritional stress:

First, stationary-phase reversions of a *lac* +1 frameshift mutation in *E. coli* (Cairns and Foster, 1991) appear to be DNA polymerase errors that escape mismatch repair. The stationary-phase reversion mechanism in the *lac* frameshift assay system is distinct from growth-dependent Lac reversion (Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996) in that the former includes homologous recombination (Harris *et al.*, 1994; 1996; Foster *et al.*, 1996) and produces mutations with a highly distinctive DNA sequence spectrum, mostly single base deletions in small mononucleotide repeats (Rosenberg *et al.*, 1994; Foster and Trimarchi, 1994). This mutation spectrum is different from growth-dependent reversions of the same allele (Rosenberg *et al.*, 1994; Foster and Trimarchi, 1994), but is identical to growth-dependent reversions in mismatch repair-null mutant strains (Longerich *et al.*, 1995). Thus, depressed mismatch repair could be responsible for the unique stationary-phase mutation spectrum. The stationary-phase mutants are not heritably mismatch repair-defective indicating that any loss of mismatch repair during stationary-phase mutation must be transient (Longerich *et al.*, 1995; Torkelson *et al.*, 1997). Such transient loss could occur either by down-regulation of the mismatch repair system, or by a block at the DNA level, for example either by under- or over-methylation of DNA sites required for operation of this methyl-directed repair system (Longerich *et al.*, 1995).

Second, the recent discovery that MutS and MutH mismatch repair protein levels decrease in stationary phase and starving bacterial cells appears to support the hypothesis of down-regulation of mismatch repair at the protein level (Feng *et al.*, 1996). However, although MutS and MutH protein levels decrease during stationary phase and starvation, so does DNA replication. Therefore, it is possible that the proper ratio of these proteins to replication errors is preserved, leaving mismatch repair functional in stationary-phase, starving cells.

If MutS, MutH, or any mismatch repair protein became limiting for mismatch repair function during stationary-phase mutation, then overproduction of the limiting mismatch repair protein might restore mismatch repair function, and thereby decrease stationary-phase mutation. We report that overproduction of MutL has this effect, that overproduction of MutS does not, and that overproduction of MutL does not act indirectly by preventing the stationary-phase decline of MutS or MutH protein levels. Overproduction of MutL does not depress growth-dependent Lac reversion. The data imply that functional MutL protein becomes limiting specifically during stationary-phase mutation, and that the decreased levels of MutS and MutH observed in stationary phase are appropriate and not limiting for the amount of DNA synthesis in stationary phase. These results suggest down-regulation of mismatch repair, at the level of MutL protein, specifically during the differentiated state of stationary phase and provide the first evidence supporting down-regulation of mismatch repair in any natural circumstance in any organism.

RESULTS

We tested whether mismatch repair proteins MutS, MutL, or both become limiting during stationary-phase reversion of a *lac* frameshift mutation in *E. coli*. If the mismatch repair proteins become limiting transiently during stationary-phase mutation, then overproduction of those proteins from plasmids might be expected to restore mismatch repair function and inhibit formation of stationary-phase Lac$^+$ revertants. To measure stationary-phase reversions of the *lac* frameshift mutation, the frameshift-bearing cells are spread on minimal lactose plates (Cairns and Foster, 1991; Harris *et al.*, 1994; 1996; see MATERIALS AND METHODS). At about two days of incubation, growth-dependent revertant colonies appear. These are followed by stationary-phase revertant colonies which accumulate during the next several days (Cairns and Foster, 1991; Foster, 1993) and which form *via* a different molecular mechanism which includes genetic recombination (reviewed by Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996).

The term "stationary-phase mutation" is used here to refer to what has also been called "adaptive mutation" (Foster, 1993). This process occurs in stressed, starving cells, and so may reflect stress responses to starvation and in general. Features of the novel recombination-dependent stationary-phase mutation mechanism in the assay system used here are seen in some (Taddei *et al.*, 1995) but not all stationary-phase mutation systems assayed (*e.g.* Hall, 1995; Galitski and Roth 1995; 1996; Foster and Trimarchi, 1995; Radicella *et al.*, 1995). For those systems with no known features distinct from growth-dependent mutation (*e.g.* Galitski and Roth, 1995; 1996; Foster and Trimarchi, 1995; Radicella *et al.*, 1995), the stationary-phase mutations may not represent a distinct process.

Overproduction of MutL Inhibits Stationary-phase Lac$^+$ Mutation Data in FIGURE 6-1 show that overproduction of MutL mismatch repair protein from a multicopy plasmid depresses stationary-phase Lac reversion by about four-fold relative to that seen with a strain carrying a control plasmid. The results of multiple experiments of this type are compiled in TABLE 6-1. The depression is seen when MutL is overproduced either alone, or in combination with MutS, and is not observed when only MutS is overproduced (FIGURE 6-1-B, TABLE 6-1). This indicates that MutL is limiting, or stabilizes another protein that is limiting, during stationary-phase mutation. The plasmid producing both MutL and MutS was used in many of the experiments reported here. However, the results in FIGURE 6-1-B and TABLE 6-1 demonstrate that overproduction of MutL alone is sufficient to depress mutation.

Overproduction of MutL Does Not Inhibit Growth-dependent Lac$^+$ Mutation To assess whether the effect of MutL overproduction on mutation is specific to stationary-phase mutation, the effects of MutL (and MutS) overproduction on growth-dependent reversion of the same *lac* frameshift allele were assessed in two ways:

First, the mechanism of stationary-phase, but not growth-dependent, Lac$^+$ reversion in these strains requires recombination genes (Harris *et al.*, 1994; 1996; Foster *et al.*, 1996) including functional *recA*. Thus, one may examine growth-dependent Lac$^+$ reversion in the absence of any contribution of the recombination-dependent mutation mechanism by using a *recA* null mutant strain. Data in FIGURE 6-1-A show that the *recA* cells overproducing MutL (and MutS) display no decrease in *recA*-independent Lac$^+$ mutation relative to cells carrying the control plasmid.

Second, data in TABLE 6-2 (experiments 1-3) show that growth-dependent Lac reversion rates are unaffected by overproducing MutL (and MutS), relative to the mutation rates in strains bearing the control plasmid.

These data appear to contrast with results from a previous study in which co-overproduction of MutS and MutL appeared to inhibit growth-dependent Lac reversion relative to a control plasmid-bearing strain (Foster *et al.*, 1996). However, in that study all growth-dependent mutants were scored after 2 days. We found that cells carrying the MutS and MutL-overproducing plasmid take longer than two days to form colonies (TABLE 6-2). Therefore we scored the growth-dependent mutants of each strain after an experimentally determined incubation-time specific for that strain (TABLE 6-2). When controlled for speed of colony formation in this way, no difference in growth-dependent reversion rates is detected between control and overproducing strains (TABLE 6-2). These results allow us to infer that mismatch repair protein MutL is limiting specifically during stationary-phase and not growth-dependent Lac reversion.

Mutants That Display Stationary-phase Hypermutation Show Greater MutL-promoted Depression of Stationary-phase Mutation The apparent deficiency of functional MutL during stationary-phase Lac reversion (FIGURE 6-1-B, TABLE 6-1) might be partial rather than absolute. If so, then strains that are hypermutable for stationary-phase Lac mutation by virtue of creating more DNA polymerase errors might reduce mismatch repair protein levels further by titrating away the limiting protein. Because stationary-phase Lac reversion uses recombination functions, whereas growth-dependent reversion does not (Harris *et al.*, 1994; 1996; Foster *et al.*, 1996), this idea can be tested using *rec* mutants that are hypermutable specifically in stationary-phase Lac reversion. A hyper-recombinagenic and stationary-phase-hypermutable *recD* mutant strain (Harris *et al.*, 1994) was used. The data in FIGURE 6-2 show that overexpression of MutL (with MutS) causes a dramatic fifteen-fold reduction of stationary-phase Lac reversion in this strain, whereas growth-dependent Lac reversion is unaffected (FIGURE 6-2-B *recA recD* results, and TABLE 6-2, experiments 4-7).

A stationary-phase-hypermutable *recG* strain (Foster *et al.*, 1996; Harris *et al.*, 1996) was affected similarly by overproduction of MutS plus MutL (Foster *et al.*, 1996, and our data not shown). The authors postulated a direct protein-protein interaction between RecG and the MutS and MutL proteins (Foster *et al.*, 1996). Our results showing a similarly large depression of stationary-phase Lac reversion by overproducing MutL and MutS in a *recD* strain argue against this interpretation because RecD acts at a very different stage in recombination, and on a different DNA intermediate than RecG does (Rosenberg and Hastings, 1991; Kowalczykowski *et al.*, 1994; Myers and Stahl, 1994; West, 1992; West, 1994). The data support the idea that the hypermutable *recD* and *recG* strains simply provide more recombination intermediates which are hypothesized to prime the DNA synthesis with polymerase errors that leads to stationary-phase Lac reversion (Harris *et al.*, 1994; 1996; Foster *et al.*, 1996). The increased errors would further reduce effective MutL levels by titration of MutL.

This latter interpretation, that MutL mismatch repair protein is only partially limiting during stationary-phase mutation, is consistent with the observation that a *mutL*-defective strain shows *recA*-dependent stationary-phase hypermutation (Harris *et al.*, 1997).

MutL Overproduction Does Not Inhibit Mutation by Preventing MutS or MutH Decline During Stationary Phase and Starvation We wished to address the possibility that MutL overproduction might inhibit stationary-phase mutation by preventing the reported declines in either MutS or MutH proteins in starving, stationary-phase cells (Feng *et al.*, 1996). Therefore, we used quantitative Western blots to determine the levels of MutL, MutS, and MutH proteins in cells overproducing MutL, MutS, and MutL plus MutS during stationary-phase starvation on lactose medium, and during growth.

The Western blots were as performed previously (Feng *et al.*, 1996) with modifications (MATERIALS AND METHODS). FIGURE 6-3 summarizes quantification of the amounts of MutL, MutS, and MutH proteins in growing and starved stationary-phase cells carrying the control and overproducing plasmids. The data are from three to five independent experiments for each determination.

The results can be summarized as follows:

First, we see that the plasmids constructed and used to overproduce MutL do so by about 20-30-fold (FIGURE 6-3-A, white bars and gray bars). Those overproducing MutS do so by 60-130-fold (FIGURE 6-3-B, striped bars and gray bars). Less than ten percent break-down products were observed in the overproducing strain (data not shown). This demonstration that MutS is overproduced allows us to rule out the possibility that the MutS plasmid did not inhibit stationary-phase mutation (FIGURE 6-1-B, TABLE 6-1) due to a failure to overproduce MutS protein. We conclude that MutS overproduction does not inhibit stationary-phase Lac reversion.

Second, in strains carrying the control plasmid, we observe declines in MutS and MutH proteins early in stationary phase, and on prolonged exposure of the *lac*⁻ cells to starvation on lactose minimal medium (FIGURE 6-3-B, C, and D, black bars). This is similar to results reported previously with plasmid-free cells (Feng *et al.*, 1996).

Third, we note that overproduction of MutS plus MutL may have a small stabilizing effect on MutH, preventing MutH decline during prolonged starvation (FIGURE 6-3-C). This can be assessed only in the experiment measuring MutH as ng/μg total cellular protein (FIGURE 6-3-C), and not by measuring MutH monomers per cell because we observe that cells overproducing MutS plus MutL are at least twice as long as normal cells (data not shown). The mechanism of this enlargement is unknown. However, it may suggest an interaction between MutS and MutL and prokaryotic cell cycle control similar to that observed with eukaryotic mismatch repair and eukaryotic cell cycle regulation (Hawn *et al.*, 1995; Anthoney *et al.*, 1996). If the increase in MutH seen in FIGURE 6-3-C is significant, then MutS plus MutL might make direct contact with MutH such that their overabundance could prevent MutH loss. If, for example, MutH were a target of a stationary phase-specific protease (Gottesman and Maurizi, 1992; Miller, 1996), it could be that contact with MutS plus MutL protects against such proteolysis. Alternatively, overproduction of MutS and MutL might titrate such a protease directly. Other explanations are possible.

Finally, neither stabilization of MutS, nor significant stabilization of MutH is seen when MutL is overproduced alone (FIGURE 6-3-B, C, and D, white bars). For MutH, two different sets of experiments are shown (FIGURE 6-3-C and D). The first, measured in ng MutH protein per 150μg total cellular protein (FIGURE 6-3-C), shows a slight but statistically insignificant trend in prevention of MutH decline by overproducing MutL. The second set is measured as numbers of MutH monomers per cell. This may be a more relevant measure, as the protein composition of cells changes dramatically in stationary phase, mostly due to loss of ribosomes (Davis *et al.*, 1986; Bremer and Dennis, 1987). These data show no significant prevention of MutH loss by MutL-overproduction (FIGURE 6-3-D, white bars). We conclude that stabilization of MutH does not correlate with the depression of stationary-phase Lac reversion which is seen in cells producing MutL alone (FIGURE 6-1, TABLE 6-1). Thus the depressing effect of MutL on stationary-phase mutation cannot be explained by stabilization of either MutS or MutH levels during starvation.

DISCUSSION

The results reported here imply that during recombination-dependent stationary-phase mutation, mismatch repair activity is diminished by a decrease in the level of functional MutL protein. The data also imply that the observed declines in MutS and MutH proteins (Feng *et al.*, 1996; FIGURE 6-3-B, C, and D) are proportional to decreased replication during starvation and stationary phase, and do not cause a loss of mismatch repair function.

Significance As far as we know, the results reported here represent the first natural circumstance in which mismatch repair activity has been shown to be limiting. This is significant because of the powerful effect of the mismatch repair system on maintenance of genetic and genomic stability in organisms from bacteria to humans. Cells that lack mismatch repair have thousand-fold higher spontaneous mutation rates (Modrich, 1991), recombine sequences of only partial identity (Rayssiguier *et al.*, 1989; Worth Jr. *et al.*, 1994; de Wind *et al.*, 1995; Matic *et al.*, 1995; Matic *et al.*, 1996; Baker *et al.*, 1995; Baker *et al.*, 1996; Chambers *et al.*, 1996; Zahrt and Maloy, 1997) causing genome rearrangements (Petit *et al.*, 1991), and manifest microsatellite instability and cancer (Modrich 1994; 1995; Radman *et al.*, 1995).

Mismatch repair-deficiency is correlated with successful bacterial pathogenesis, implying that a mutator phenotype is selected in the war between pathogens and the host immune system (LeClerc *et al.*, 1996). But because the majority of the successful pathogens examined were not heritably mismatch repair-defective, these may have succeeded by a transient mismatch repair-deficiency.

Mismatch repair also prevents interspecies recombination (Rayssiguier *et al.*, 1989; Matic *et al.*, 1995; 1996; Hunter *et al.*, 1996; Zahrt and Maloy, 1997), and its component proteins participate in transcription-coupled repair of damaged DNA (Mellon and Champe, 1996), and in very-short-patch repair (Lieb, 1987; Jones *et al.*, 1987a; Jones *et al.*, 1987b; Raposa and Fox, 1987; Zell and Fritz, 1987). Diminished mismatch repair in response to environmental signals would mean that mutation, improper recombination, genome rearrangements and genetic instability might vary in response to a cell's environment. This would have profound consequences for evolution, development, microbial pathogenesis, cancer formation, tumor progression and acquisition of drug-resistance in tumors and pathogens.

Mechanism The lack of functional MutL implied by the data reported here cannot be attributed simply to a decrease in the amount of MutL protein in stationary-phase, as MutL levels do not appear to change during stationary phase and starvation (Feng *et al.*, 1996; FIGURE 6-3-A). Several explanations for functional MutL deficiency are possible.

First, MutL protein might be modified or processed in stationary phase to a non-functional form.

Second, though present in abundant quantity, MutL might become limiting by means of titration by a DNA substrate formed during stationary-phase. Creation of such a substrate could be a regulation mechanism. If it occurs, it is demonstrably a natural part of starvation, and not artificially induced in this system.

Mismatch repair can be saturated artificially: by excess polymerase errors of a proofreading-defective mutant DNA polymerase (Damagnez *et al.*, 1989; Schaaper and Radman, 1989), by mutagens thought to increase polymerase error (Cupples *et al.*, 1990), or by overproduction of single-strand DNA with regions of secondary structure containing mismatched bases (Maas *et al.*, 1994; 1996). Thus, DNA substrates can titrate mismatch repair proteins in growing cells. Overproduction of a protein that interacts with MutL may as well (Doiron *et al.*, 1996). The limiting proteins titrated were MutL or MutH (Schaaper and Radman, 1989) or MutS alone (Maas *et al.*, 1996). Titration of MutL is compatible with the results of Schaaper and Radman (1989) but is not obviously so with those of Maas *et al.* (1996). The titration hypothesis need not conflict with the apparent abundance of MutL and scarcity of MutS and MutH in stationary-phase, for MutL may be used as an expendable rather than a catalytic component of the reaction (Schaaper and Radman, 1989). If so, spent MutL protein might be visible on Western blots though useless to the cell for mismatch repair. Along these lines, the down-regulation of a protein that rejuvenates used MutL would also be compatible with our results.

Third, overproduction of MutL might stabilize a mismatch repair protein, other than MutS or MutH, which normally declines in stationary phase.

Fourth, recent results indicate that stationary-phase Lac reversion in the system used here occurs as part of genome-wide hypermutation in a subpopulation of the cells exposed to starvation (Torkelson *et al.*, 1997). The size of the mutagenic subpopulation was estimated to be between 10^{-4} and 10^{-5} of all of the cells starved on lactose. Thus, MutL levels might decline only in cells of the subpopulation, which would be undetectable in Western analyses of the whole population.

Is the mechanism of MutL dysfunction a regulated response or, in the case of the fourth hypothesis, might it represent random loss of MutL protein in the subpopulation? One non-random aspect of the results is that only MutL (not other mismatch repair proteins) appears to become limiting. Ninio suggested that the normal error rates of replication, transcription, and translation should lead to transient and heritable mutator subpopulations, for example by faulty synthesis of (MutL or) any protein involved in replication fidelity (Ninio, 1991). Ninio's model predicts frequencies of heritable mutator mutants to be found amongst cells carrying mutations that are far greater than those observed in this system (Torkelson *et al.*, 1997), suggesting the possibility that the mutator state is not random, but rather a program. However the error rates used in Ninio's calculations may not apply during starvation. Whether the loss of MutL function is accidental or programmed, it occurs in response to environmental conditions. Environmental influence over genetic stability could be important for reasons discussed above.

Implications for Stationary-phase Mutation Bacteria differentiate and execute specific developmental programs to deal with stationary phase and starvation (Seigele and Kolter, 1992; Kolter *et al.*, 1993) during which they generate special mutants with the ability to prevail under limiting conditions (Zambrano and Kolter, 1996). The stationary-phase mutation-specific loss of mismatch repair function reported here could be part of a developmental program for generating such mutants.

Stationary-phase reversions of the *lac* frameshift mutation in the system used here have been shown to result from a genome-wide hypermutable state in a subpopulation of the starved cells (Torkelson *et al.*, 1997). MutL-deficiency might or might not be the special feature that makes the subpopulation different. That is, the whole population might be MutL-deficient but only the subpopulation might, for example, perform the recombination necessary for Rec-dependent stationary-phase mutation (Rosenberg, 1994; Rosenberg *et al.*, 1995; 1996).

Also, (Torkelson *et al.*, 1997) stationary-phase mutations are found not to be directed, in a Lamarckian manner, to the gene under selection (*lac*) but rather occur in multiple unselected genes in all replicons in the cell [see also (Foster, 1997) for evidence of unselected mutation]. This supports Darwinian models for stationary-phase mutation that include random mutation followed by selection for the adaptive mutation. However, the implication of the findings reported here, that mutation rates could be altered significantly by decreasing mismatch repair in response to environmental cues, suggests that the cells may generate the variation upon which selection acts more vigorously when they "need" to evolve. Where such a mutagenic mode fits in the continuum between Lamarckian and Darwinian mutation models will probably be a subject of continuing discussion. Down-regulation of mismatch repair could provide a molecular mechanism for achieving rapid genetic change when selection is present. On the microscopic scale of changes in bacterial genotype, this could contribute to mechanisms producing the punctuations in punctuated equilibria (Eldredge and Gould, 1972).

MATERIALS AND METHODS

Plasmids Plasmids were modified from pBR322 (Bolivar *et al.*, 1977) to carry kanamycin-resistance (Kan^r) (pSL4) and the following *E. coli* genes regulated by their natural promoters: *mutL* (pSL5); *mutS* (pSL6); *mutS* and *mutL* (pSL7). These genes are overexpressed due to the high copy number of pBR322. In pSL4 the *Hin*dIII-*Bam*HI fragment of pBR322 is replaced by the Kan^r-conferring *Hin*dIII-*Bam*HI fragment of pKC31 (RN Rao, described by *e.g.* Rosenberg, 1988). pSL5 contains a *Pst*I-*Hin*dIII fragment of pAL51 carrying *E. coli mutL* (Lu *et al.*, 1984) and the *Hin*dIII-*Bam*HI fragment of pKC31 replacing the *Pst*I-*Bam*HI fragment of pBR322. In pSL6 the pBR322 *Cla*I-*Bam*HI fragment is replaced by the *mutS*-containing *Cla*I-*Hin*dIII fragment of pMS312 (Su and Modrich, 1986) and the *Hin*dIII-*Bam*HI fragment of pKC31. In pSL7 the *Cla*I-*Pst*I fragment of pBR322 is replaced by the *Cla*I-*Bgl*II fragment of pSL6, the *Bam*HI-*Hin*dIII fragment of pKC31, and the *Hin*dIII-*Pst*I fragment of pAL51. All plasmid genotypes were confirmed by restriction mapping and by complementation of the mutator phenotype (assayed as by Longerich *et al.*, 1995; Torkelson *et al.*, 1997) of *mutS* and/or *mutL*-defective *E. coli* strains.

Lac$^+$ Mutation Assays For Lac$^+$ frameshift reversion studies, derivatives of a strain carrying the *lacI33* allele (Cairns and Foster, 1991) and carrying the plasmids described above were used. This strain is deleted for the chromosomal *lac* operon and bears an F' episome carrying a *lacI-lacZ* fusion gene with a +1 frameshift mutation in *lacI* which is polar on *lacZ* (Cairns and Foster, 1991). Procedures for measurement of stationary-phase Lac$^+$ mutation, and for measurements of growth-dependent Lac$^+$ mutation rates were modified from (Harris *et al.*, 1996) as follows: 50μg/ml kanamycin were included in the minimal glycerol broth and 5μg/ml kanamycin in the minimal lactose plates and top agar. The scavenger cell strain is as before except that it carries the kanamycin resistance-conferring control plasmid pSL4.

Western Analyses For measuring protein levels during starvation on lactose medium, it is important that Lac$^+$ revertants do not accumulate in the population assayed. Thus, the strain background analyzed was FC29 (Cairns and Foster, 1991) which is similar to the *lac* frameshift-bearing strain used but is deleted for *lac* and so is nonrevertible. Derivatives of this strain carrying the plasmids described here were used. Western analyses were as described (Feng *et al.*, 1996) with the following changes: (i) Bacteria were grown in media as described above for Lac reversion studies, washed, then spread on lactose 5µg/ml kanamycin plates as described above, but with no top agar. Plates were incubated for 8 days at 37°C. Every second day cells were washed off 10 plates with M9 salts and protein samples were prepared. Exponential and Day 0 samples were prepared using liquid cultures at a density of about 30 Klett units, and the saturated culture, respectively; (ii) MutS and MutH antisera were affinity-purified: 1ml columns containing about 2 mg His_6-MutS or His_6-MutH (Feng and Winkler, 1995) coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden), prepared according to instructions (Pharmacia), were washed sequentially with 15ml 6M guanidine HCl, 25ml Buffer A (50mM Tris-HCl, pH 7.4), 25ml Buffer B (Buffer A + 4.5M $MgCl_2$ + 1.0 mg/ml BSA), then equilibrated with 50ml Buffer A. 10ml antisera were run through the columns which were washed with 40ml 1M guanidine HCl, 20ml Buffer A and eluted with 10ml Buffer B. Elutions were dialyzed against 3L PBS, then 3L PBS + 35% glycerol. MutL antiserum was a gift from P. Modrich (Duke University).

The absolute amount of MutH per cell has been recalibrated with respect to previous experiments (Feng *et al.*, 1996) based on our finding that the standard MutH protein preparation to which calibration was performed previously had aggregated when the His_6 affinity tag was cleaved off MutH. This aggregation was not detected until later experiments in which large amounts of standard were prepared. The new estimate for MutH in cells growing exponentially in EMMG medium (Feng *et al.*, 1996) is 34 ± 7 monomers per cell.

Table 6-1. MutL overproduction diminishes stationary-phase mutation.

Mismatch repair protein overproduced from plasmid	Expt. number	Cumulative number of Lac⁺ colonies by Day 5 per 10^8 viable cells[a] (mean ± SEM)[b]	Decrease in stationary-phase mutation relative to control plasmid-bearing strain: Within each expt.	Decrease in stationary-phase mutation relative to control plasmid-bearing strain: Average (mean ± SEM)
None (Control Plasmid)	1	3.3 ± 0.2	1	1
	2	11 ± 2.1	1	
	3	19 ± 1.3	1	
	4	5.8 ± 1.9	1	
	5	89 ± 8.6	1	
	6	15 ± 1.2	1	
	7	23 ± 2.6	1	
	8	18 ± 2.2	1	
	9	37 ± 3.6	1	
MutL	5	14 ± 2.0	6.4	4.0 ± 0.7
	6	4.1 ± 0.4	3.7	
	7	7.5 ± 0.7	3.1	
	8	8.0 ± 0.7	2.3	
	9	7.9 ± 0.8	4.7	
MutS	5	56 ± 5.2	1.6	1.3 ± 0.2
	6	11 ± 2.0	1.4	
	7	20 ± 2.4	1.2	
	8	23 ± 2.3	0.8	
	9	22 ± 5.8	1.7	
MutL & MutS	1	1.1 ± 0.2	3.0	3.9 ± 0.7
	2	1.8 ± 0.4	6.1	
	3	3.2 ± 0.8	5.9	
	4	1.8 ± 0.2	3.2	
	5	15 ± 1.7	5.9	
	6	3.7 ± 0.5	4.1	

TABLE 6-1 contd.

MutL & MutS	7	19 ± 1.9	1.2
	9	20 ± 2.2	1.9

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[a] In each experiment the mean number of Lac⁺ colonies was determined from 8 to 12 independent cultures of each strain. Jackpots of growth-dependent mutants were excluded from the calculations.

[b] SEM, one standard error of the mean.

Table 6-2. Mismatch repair proteins are not limiting during growth-dependent Lac$^+$ mutation.

Relevant genotype[a]	Number of hours to form a colony[b]	Expt.	n[c]	Mutation rate[d] (Lac$^+$ cell^{-1} generation^{-1}) x10^{-10}	Mean ± S.D.
rec$^+$ [pControl]	52	1	40	3.0	8.0 ± 5.6
	56	2	40	7.0	
	67	3	40	14	
rec$^+$ [pMutSL]	71	1	40	11	8.9 ± 1.9
	58	2	39	7.2	
	67	3	40	8.6	
ΔrecA [pControl]	64	1	40	3.0	2.5 ± 1.4
	55	2	40	0.98	
	67	3	40	3.6	
ΔrecA [pMutSL]	72	1	32	5.8	5.4 ± 0.6
	58	2	40	5.8	
	67	3	40	4.7	
recD [pControl]	54	4	40	16	19 ± 11[e]
	62	5	40	11	
	52	6	40	34	
	52	7	40	13	
recD [pMutSL]	nd	4	nd	nd	13 ± 2.5[e]
	70	5	40	9.6	
	61	6	31	14	
	77	7	40	14	
recD ΔrecA [pControl]	54	4	40	6.2	5.3 ± 0.7[e]
	58	5	40	4.5	
	53	6	40	5.1	
	69	7	40	5.3	
recD ΔrecA [pMutSL]	60	4	40	5.7	9.6 ± 7.1[e]
	61	5	40	2.8	
	58	6	38	11	
	79	7	40	19	

TABLE 6-2 contd.

[a] See MATERIALS AND METHODS for names and constructions of plasmids.

[b] Determined with 10-12 different Lac$^+$ revertants of each genotype (except in expt. 4 in which 6 different revertants were used) as a t_{50}, the time at which half of the colony forming units have produced visible colonies under experimental conditions.

[c] Number of independent cultures.

[d] Growth dependent Lac$^+$ reversions were measured (Harris *et al.*, 1996) and mutation rates calculated by the method of the median (Lea and Coulson, 1949; von Borstel, 1978). nd, not done.

[e] The *recD* strain displays stationary-phase Lac$^+$ hypermutation (Harris *et al.*, 1994) and also appears hypermutable in growth-dependent Lac$^+$ reversion here. The apparent elevation of growth-dependent mutation appears to be due to spill-over of post-plating, RecA-dependent stationary-phase revertants in the hypermutable *recD* strain into growth-dependent revertant colony counts. This is implied by the finding that the increase in *recD* is entirely *recA*$^+$-dependent (experiments 4-7).

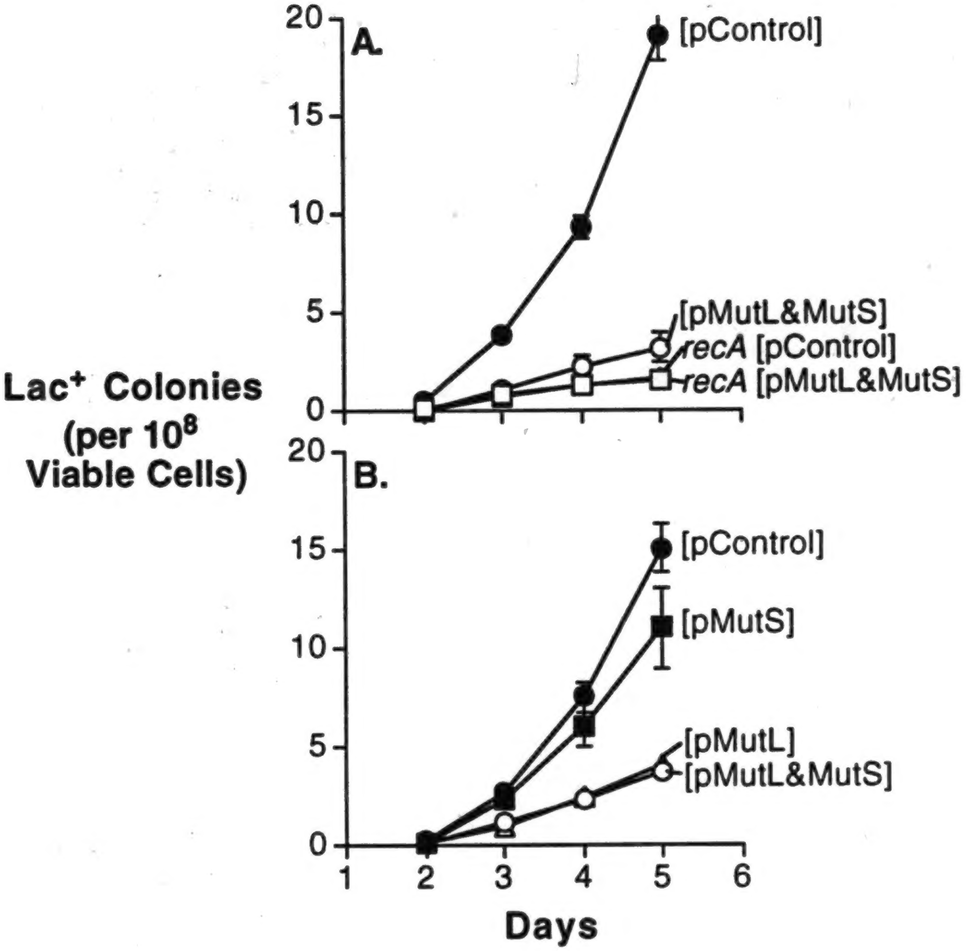


FIGURE 6-1. Overproduction of MutL alone, or with MutS, depresses stationary-phase Lac⁺ reversion. Plasmids [pControl], [pMutL&MutS], [pMutS], and [pMutL] are pSL4, pSL7, pSL6, and pSL5, respectively (MATERIALS AND METHODS). Error bars represent one standard error of the mean (SEM) and are smaller than the data point where not visible.

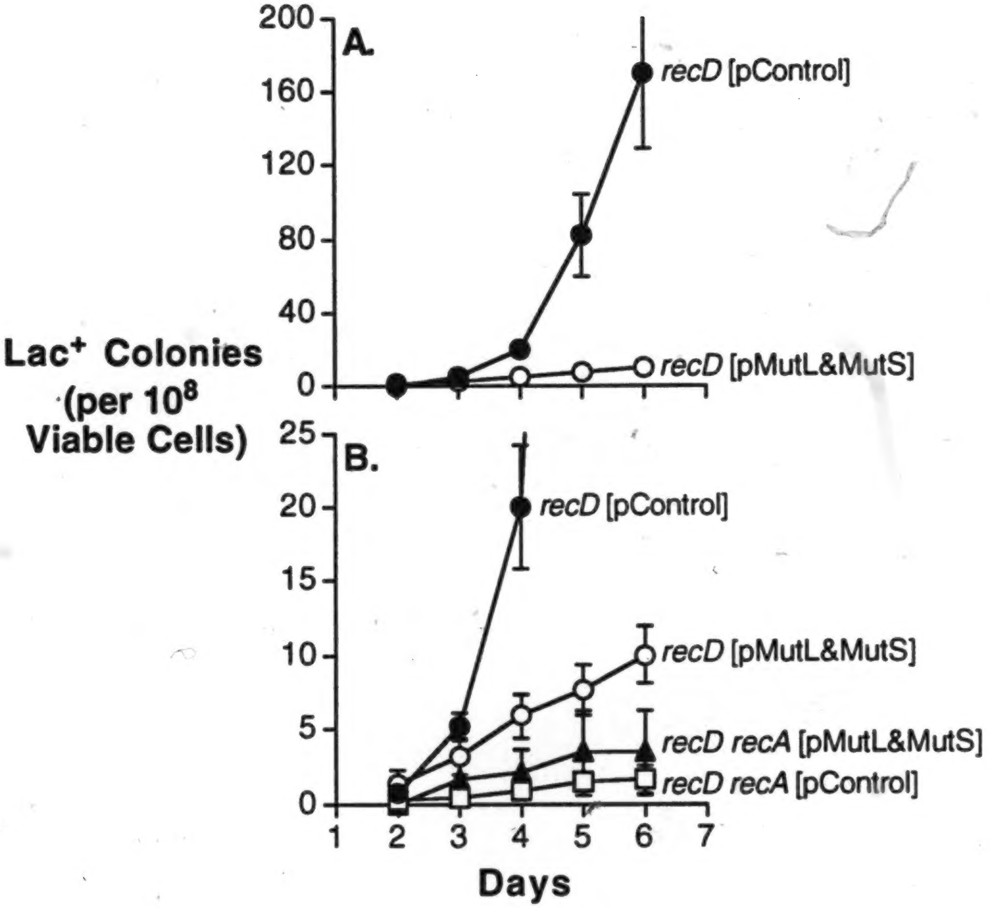
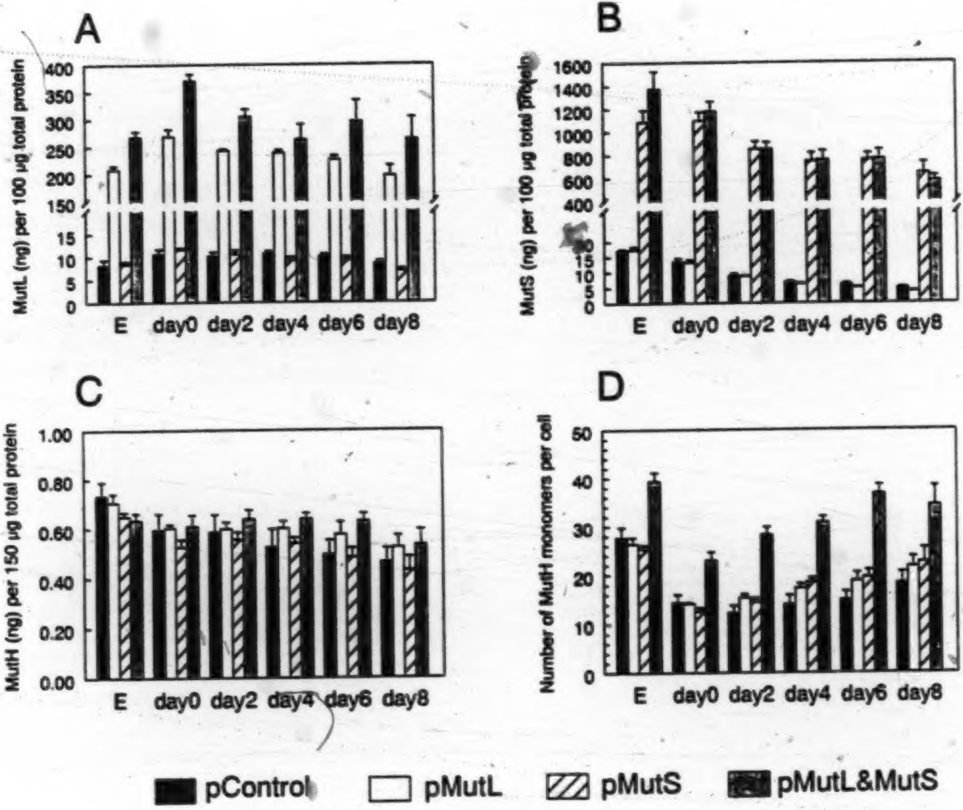


FIGURE 6-2. Inhibition of stationary-phase Lac⁺ reversion in a hypermutable *recD* strain. Plasmids [pControl], and [pMutL&MutS] are pSL4, and pSL7, respectively (MATERIALS AND METHODS). Error bars as in FIGURE 6-1.

FIGURE 6-3. Amounts of MutL, MutS, and MutH proteins in growing, stationary-phase, and starved cells carrying MutL and MutS overproducing plasmids. Data are summaries of quantifications from Western blots performed as in (Feng *et al.* 1996, see MATERIALS AND METHODS). Days 0-8 indicate days after plating the stationary-phase *lac*⁻ cells on lactose medium (MATERIALS AND METHODS). At least three experiments were performed. Each histogram bar represents the mean (error bars, SEM). **A.** MutL levels. **B.** MutS levels. For MutH, two different sets of experiments are shown. **C.** The first was measured in ng MutH protein per 150μg total cellular protein. **D.** The second set was measured as numbers of MutH monomers per cell [see MATERIALS AND METHODS for the recalibration of the number of MutH monomers per cell with respect to previous results (Feng *et al.* 1996)]. Discussed in text.


A
MutL (ng) per 100 µg total protein
400
350
300
250
200
150
15
10
5
0
E
day0
day2
day4
day6
day8
B
MutS (ng) per 100 µg total protein
1600
1400
1200
1000
800
600
400
20
15
10
5
0
E
day0
day2
day4
day6
day8
C
MutH (ng) per 150 µg total protein
1.00
0.80
0.60
0.40
0.20
0.00
E
day0
day2
day4
day6
day8
D
Number of MutH monomers per cell
50
40
30
20
10
0
E
day0
day2
day4
day6
day8
pControl
pMutL
pMutS
pMutL&MutS

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CHAPTER 7

SUMMARY AND DISCUSSION

SUMMARY

The dogma that all mutations occur randomly, during growth, and without regard for their immediate utility was challenged by the discovery of mutations that were detected only in genes whose functions were selected, occur in apparently non-dividing cells, and occur apparently in response to a non-lethal genetic selection (Ryan, 1952; 1955; Ryan and Wainwright, 1954; Shapiro, 1984; Cairns *et al.*, 1988; Hall, 1988; 1990; 1991; 1992; Cairns and Foster, 1991; reviewed by Foster, 1993; but see Foster, 1997 and Torkelson *et al.*, 1997). These discoveries, particularly those by Cairns *et al.* (1988), provoked commentary suggesting that these so-called adaptive mutations are artifacts attributable to, for example, growth, death, phenotypic lag, and/or selection (Cairns, 1988; 1990; Danchin, 1988; Grafen, 1988; Holliday and Rosenberger, 1988; Partridge and Morgan, 1988; Van Valen, 1988; Lenski *et al.*, 1989; Symonds, 1989; Lenski and Mittler, 1993; Mittler and Lenski, 1990). That is, that adaptive mutations are cryptic, growth-dependent mutations. One way to address this possibility is to distinguish between adaptive and growth-dependent mutation by elucidating a molecular mechanism of adaptive mutation.

The data in this thesis demonstrate unambiguously that adaptive reversion of a *lac* frameshift mutation in *Escherichia coli* is different from normal, growth-dependent reversion of the same mutation in the following ways:

First, adaptive reversion requires RecA and RecBC(D), two early players in the major pathway for homologous recombination in *E. coli* (CHAPTER 2, Harris *et al.*, 1994). Absence of RecD, an inhibitor of the RecBC recombinase, elevates both recombination and adaptive mutation (CHAPTER 2, Harris *et al.*, 1994). Neither affects growth-dependent reversion. These results indicate that RecABC(D)-dependent homologous recombination is a part of the molecular mechanism of adaptive Lac reversion. This was the first molecular handle on adaptive mutation (see perspective by Thaler, 1994).

Second, in contrast to their apparent redundancy in homologous recombination (Lloyd, 1991), the RuvABC and RecG resolution systems play opposing roles in Lac$^+$ adaptive mutation: RuvABC is required, whereas RecG inhibits reversion of the *lac* frameshift mutation (CHAPTER 3, Harris *et al.*, 1996; see also Foster *et al.*, 1996). Temporary absence of both resolution systems elevates mutation (CHAPTER 3, Harris *et al.*, 1996) suggesting that recombination intermediates themselves promote adaptive reversion. Neither system affects growth-dependent reversion.

Third, DNA polymerase III (PolIII) plays a direct role in Lac$^+$ adaptive mutation, as a PolIII antimutator allele decreases adaptive Lac reversion even in mismatch repair-defective cells (CHAPTER 4, Harris *et al.*, 1997a; see also Foster *et al.*, 1995). This discovery was foreshadowed by the demonstration that the adaptive mutant sequences are mostly -1 deletions in mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg *et al.*, 1994), which are common DNA polymerase mistakes (Ripley, 1990). This unique mutation spectrum also suggests that such errors persist *via* mismatch repair dysfunction (Rosenberg *et al.*, 1994; Longerich *et al.*, 1995). The mismatch repair dysfunction must be transient because most Lac$^+$ adaptive mutants are not heritably mutator (CHAPTER 5; Longerich *et al.*, 1995; Torkelson *et al.*, 1997).

Fourth, mismatch repair activity appears to be transiently limiting during Lac$^+$ adaptive mutation, at the level of MutL protein (CHAPTER 6, Harris *et al.*, 1997b). MutL overproduction decreases Rec-dependent-, but not growth-dependent, *lac* frameshift-reversion. Measurements of mismatch repair protein levels in starved, MutL-overproducing cells show that neither MutS or MutH levels, which decrease during starvation (Feng *et al.*, 1996), are stabilized by MutL overproduction. These results indicate that MutL, or a protein with which MutL associates, other than MutS or MutH, becomes limiting during Rec-dependent Lac$^+$ adaptive mutation. Also, the data imply that the starvation-associated declines of MutS and MutH are proportional to the decrease in

DNA replication that occurs in starved, stationary-phase cells. These results represent the first natural circumstance in which mismatch repair activity has been shown to become limiting.

The results in this thesis elucidate a novel molecular mechanism of mutation in *E. coli* involving homologous recombination, DNA synthesis, and transient mismatch repair-suspension.

DISCUSSION

Is Adaptive Mutagenesis Directed to the Selected Gene? Two recent studies using the *lac*-frameshift assay system demonstrate that it is not. First, we showed that unselected mutations occur in chromosomal, F' episomal, and pBR322-located genes in Lac$^+$ adaptive mutants, but not in starved or unstarved Lac$^-$ cells (Torkelson *et al.*, 1997). That these unselected mutations occur *via* the same recombination-dependent mechanism as Lac$^+$ adaptive mutations is supported by the finding that unselected reversions of a +1 frameshift mutation in the *tet* gene of pBR322 are -1 deletions in mononucleotide repeats (Torkelson *et al.*, 1997) like Rec-dependent reversions of the *lac* frameshift mutation (Foster and Trimarchi, 1994; Rosenberg *et al.*, 1994). The unselected mutations are prevalent among Lac$^+$ revertants but not in the whole population of *lac*$^-$ cells starved on lactose. These data imply that only a subpopulation of cells is mutable and that genome-wide hypermutation in this subpopulation of starved, stationary-phase cells underlies Lac$^+$ adaptive mutation. Second, unselected, Rec-dependent frameshift mutations in a modified Tn*10 tet* gene on the F' occur in starved *lac* frameshift-bearing cells during Lac$^+$ adaptive mutation (Foster, 1997). These data show that Rec-dependent mutation is not adaptive in that only selected mutations accumulate. Thus, "adaptive", which was used to describe these mutations, may be misleading. Stationary-phase" (Ryan and Wainwright, 1954) may be more appropriate for describing mutations that occur in non-growing or slowly-growing cells under non-lethal genetic selection.

Differentiated-subpopulation Model Models for the molecular mechanism for stationary-phase mutation in the *lac* frameshift assay system must include the following molecular features:

(i) Proteins of the RecBCD recombination pathway are involved: RecA (CHAPTER 2, Cairns and Foster, 1991; Foster, 1993; Harris *et al.*, 1994), RecBC(D) (CHAPTER 2, Harris *et al.*, 1994), and RuvABC (CHAPTER 3, Foster *et al.*, 1996; Harris *et al.*, 1996);

(ii) DNA double-strand breaks (DSBs) are implicated as molecular intermediates (CHAPTER 2, Harris *et al.*, 1994), because DNA DSBs are the only known DNA access points for RecBCD (Kowalczykowski *et al.*, 1994; Myers and Stahl, 1994);

(iii) involvement of recombination intermediates (CHAPTER 3, Harris *et al.*, 1996);

(iv) mistakes made by DNA PolIII (CHAPTER 4, Foster *et al.*, 1995; Harris *et al.*, 1997a);

(v) transiently diminished mismatch repair (CHAPTERS 5 & 6, Harris *et al.*, 1997b);

(vi) de-repression of one or more genes of the LexA-regulon (APPENDIX II; also see Foster *et al.*, 1996); and

(vii) transfer genes of the F' episome (Foster and Trimarchi, 1995; Galitski and Roth, 1995; Radicella *et al.*, 1995).

Models must also account for data showing that some sites are hot for Rec-dependent mutation [*e.g. lacI33-lacZ* (Cairns and Foster, 1991; Harris *et al.*, 1994) and *tetA* (Foster, 1997) on the F' episome], whereas others are not [*e.g. rpoB* (Foster, 1994) and *lacI33-lacZ* (Radicella *et al.*, 1995) at their normal chromosomal locations], and for data showing that unselected, Rec-dependent mutations occur in stationary-phase, starved cells (Foster, 1997; Torkelson *et al.*, 1997). Also, models must accommodate data showing that unselected mutations occur in all replicons (Torkelson *et al.*, 1997), and that a hypermutable subpopulation generates Rec-dependent mutation - unselected mutations occur at high frequencies genome-wide in Lac$^+$ revertants (Torkelson *et al.*, 1997; model suggested by Hall, 1990).

These results are assembled in the differentiated-subpopulation model in FIGURE 7-1 (some aspects were proposed originally by Hall, 1990; Harris *et al.*, 1994 and developed progressively by Rosenberg, 1994; Rosenberg *et al.*, 1994; 1995; 1996; Longerich *et al.*, 1995; Harris *et al.*, 1996; 1997a; 1997b; Torkelson *et al.*, 1997). Stress of a non-lethal genetic selection is proposed to cause a subpopulation of cells to enter a hypermutable state in which DNA DSBs occur. RecABC-mediated recombination at DNA DSBs creates recombination intermediates that prime PolIII-dependent DNA synthesis. Mistakes made during such DNA synthesis may persist as mutations due to mismatch repair dysfunction. The LexA-repressed gene product(s) involved in stationary-phase Lac reversion (APPENDIX II) could work at any stage of this model. The hypermutable subpopulation is proposed to mutate until a mutation(s) allows the cell to overcome the selective pressure.

Cell death was an important component of previous models (*e.g.* Hall, 1990; Rosenberg *et al.*, 1995) required to explain the inability to detect unselected mutations (Hall, 1990; Foster, 1994). This component is now unnecessary because unselected, Rec-dependent mutations occur in stationary-phase, starved *lac* frameshift-bearing cells (Foster, 1997; Torkelson *et al.*, 1997).

The mutability of some loci and not others may be due to two non-exclusive reasons: First, proximity to a DSB site may be necessary. The non-mutability of the *lac* frameshift mutation at its normal chromosomal location (Radicella *et al.*, 1995) may be because this locus is far from a DSB site (Rosenberg *et al.*, 1995). The Tra proteins offer a route to DSB formation on the F'. Single-strand nicks made at *oriT* could lead to DSBs [discussed in CHAPTER 3; see also (Rosenberg *et al.*, 1995; Torkelson *et al.*, 1997)]. Chromosomal regions that display a high incidence of DSBs, such as the replication terminus (Louarn *et al.*, 1994), might mark spots for Rec-dependent adaptive mutation (Harris *et al.*, 1994). Second, the Rec-dependent mutational mechanism may be frameshift-specific. *rpoB* mutations, which confer rifampicin resistance, are not elevated during Lac$^+$ adaptive mutation (Foster, 1994). This may be because such mutations are mostly base substitutions (Rangarajan *et al.*, 1997). *rpoB* also may be far from a DSB site.

Future Direction The novel mutational mechanism described here is different from that occurring during growth. This highlights the need to abandon dogmata in which spontaneous mutations occur uniformly in dividing cells. Stationary-phase mutation could be important in evolution because a capacity to make mutations in response to stress would provide cells with a selective advantage. Such mutations may be a source of punctuations in the punctuated equilibria theory (Eldredge and Gould, 1972). The generality of the stationary-phase mutation mechanism described here awaits testing, as does elucidation of mutational mechanisms underlying cancer, development, and evolution which may have features in common with the mechanism described here.

STRESS (*e.g.* starvation)

⇓

SUBPOPULATION OF STATIONARY-PHASE STARVED
CELLS ENTERS HYPERMUTABLE STATE

⇓

DSBs

⇓

- RECBC(D)-MEDIATED HOMOLOGOUS RECOMBINATION
- DNA SYNTHESIS
- DNA POLYMERASE ERROR (DNA POLYMERASE III IMPLICATED)
- TRANSIENT MISMATCH REPAIR DEFICIENCY

⇓

MUTATION

⇓

STRESS AND HYPERMUTATION STOP

⇓

GROWTH AND CELL DIVISION RESUME

FIGURE 7-1. The differentiated-subpopulation model for recombination-dependent stationary-phase reversion of the *lac* frameshift mutation. Discussed in text. See also Torkelson *et al.* (1997).

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APPENDIX I

A COMPLETE LIST OF *E. COLI* K-12 STRAINS AND PLASMIDS USED, CONSTRUCTED OR BROUGHT TO THE LAB BY REUBEN S. HARRIS

INTRODUCTION

This appendix lists all of the *Escherichia coli* strains (TABLE I-1) and plasmids (TABLE I-2) that were used, constructed or brought to the lab by Reuben S. Harris (RSH). Also included are strains constructed by undergraduate research students Cindy Wong, Kimberly J. Ross and Roger Sidhu under the direction of RSH. All strains were assigned a Susan M. Rosenberg (SMR) collection number. Those constructed by members of the SMR lab are named after that individual and numbered accordingly. The numbers begin at 1 (*e.g.* SMR666 = RSH1) or correspond to the SMR number for that strain (*e.g.* SMR577 = HR577). For some constructions more than one isolate has been constructed independently and entered in the SMR collection - these instances are noted.

The genetic nomenclature for *E. coli* used here is that used by the *E. coli* Genetic Stock Center (CGSC) (Bachmann, 1996; Berlyn *et al.*, 1996; summarized neatly by Stewart, 1995).

Standard bacteria and bacteriophage protocols were employed throughout (Miller, 1992). Each construction is described with standard symbols and abbreviations (see TABLE I-3) and includes the following information in this order: (i) the procedure used, together with the bacteria, bacteriophages and or plasmids involved; (ii) the selection employed; (iii) the phenotypes screened; and (iv) brief notes and/or references. Most strains were constructed by P1-mediated transduction of a selectable genetic marker. For example, SMR1551 was constructed by transducing SMR506 with P1 grown on SMR603, selecting Tet^r and screening for sensitivity to UV [SMR506 x P1(SMR603); Tet^r; moderately UV^s; NOTE: identical to SMR1983 (reference)]. For transductional and conjugational crosses the recipient is always denoted first. λ genotypes and tricks are included in the LEGEND. Some of the common tests used to verify the genotypes of newly constructed strains include:

(i) The UV strip test: Saturated liquid cultures of the strains to be tested were spread in a line with a capillary tube across the surface of an agar plate, allowed to dry, and irradiated in cross-sections using increasing UV intensities (joules/m^2). Overnight incubation allowed survivors to grow. This test was used to confirm the presence (or absence) of mutations that confer a characteristic UV sensitivity (denoted as UV^s or UV^r; *e.g.* a *recA* mutant SMR2001: extremely UV^s).

(ii) The mutator test: Mutation to Lac$^+$, Rif^r, or Nal^r was scored as follows: If to Lac$^+$, 10μl of a saturated, minimal M9 glycerol culture were spotted to a minimal M9 lactose plate, spread into a 2-3 cm wide circle, and incubated 2 or 3 days before scoring Lac$^+$ mutants. If Rif^r or Nal^r was selected, 100μl (or more) of a saturated overnight culture were spread onto the appropriate selective media and resistant colonies were scored after a 24 hour incubation. Alternatively, for Nal^r selections, cells were spotted as described for mutation to Lac$^+$, but to a non-selective, rich medium, upon which a small amount of nalidixic acid powder was placed. These plates were incubated overnight. The number of Nal^r colonies in the zone of clearing, which was created by diffusion of the nalidixic acid, were scored. Mutators, *e.g.* a mismatch repair-deficient *mutS* strain, often displayed 10-fold, or more, mutation than nonmutators [denoted as "mutator (phenotype scored)"; *e.g.* SMR3404: strong mutator (Lac$^+$)].

(iii) The RecD$^-$ test: Inactivation of *recD* allows rolling circle replication of λ *red gam* mutants. This can be monitored using the following λ plaque assay: A λ *red gam* that lacks χ forms small plaques on a *recD*$^+$ strain and large plaques on a *recD*$^-$ strain, whereas an identical λ, except χ^+, forms big plaques on both hosts [together denoted as the phenotype RecD$^-$; *e.g.* SMR692].

(iv) The F episome test: A number of bacteriophage are specific for a particular sex of host. Strains harboring an F (or F') episome (males) are sensitive the male-specific phage R17, but insensitive to the female-specific phage T7. The opposite is true for F$^-$ strains (females). The presence (or absence) of F was determined by spotting 10^5-10^7 phage onto a lawn of bacteria which were spread on an agar plate (minimal or rich). Overnight incubation revealed the sex of the bacteria [denoted as phage^s or phage^r; *e.g.* SMR2027: R17^s].

TABLE I-1. ***E. coli*** **K-12 strains.**

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
74	SMR74	C600 *dam13*::Tn*9*	Lab collection
85	SMR85	*recA801* (=*srfA801*) *srl-300*::Tn*10*	Lab collection
91	SMR91	*mutL211*::Tn*5*	"
121	JC9387	Su$^-$ AB1157 *recB21 recC22 sbcB15 sbcC201*	A.J. Clark
122	JC11450	AB1157 spontaneous Su$^-$	A.J. Clark
123	JC11451	Su$^-$ *his*$^+$ *sbcB15*	A.J. Clark
124	FS2504	*recJ284*::Tn*10*	Lab collection (Lovett and Clark, 1984)
127	C600	*thr-1 leuB6 lacY1 supE44*(=SuII$^+$) *rfbD1 thi-1 mcrA1 fhuA21*	Lab collection; also referred to as CR34 (Bachmann, 1996)
147	YK1100	W3110 *trpC9941*	Lab collection
160	SMR160	*polA12*(Ts) *rha lac ilv*	Lab collection; **NOTE:** requires only valine, not isoleucine
165	JC9388	Su$^-$ AB1157 *recB21 recC22 sbcA*	Lab collection
235	JAS7	*recA200*(Ts) *srl-300*::Tn*10*	Lab collection
272	JC17101	*recA803* (=*srfA801*) *srl-300*::Tn*10*	A.J. Clark
308	SMR308	C600 *mutL218*::Tn*10*	Lab collection
346	SMR346	C600 *mutL211*::Tn*5*	"
362	SMR362	594 *mutU*::Tn*5* Str^r	Lab collection
390	SMR390	594 *mutS201*::Tn*5*	"
417	SMR417	*trp*::Tn*5*	"

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
423	SMR423	C600 SuIII hsdrK$^-$ hsdrK$^+$ *trp*::Tn*5* *recD1903*::Tn*10*	Lab collection; **NOTE:** this strain has been repurified & designated SMR3578 (Harris *et al.*, 1997c; APPENDIX IV)
438	SMR438	*mutS201*::Tn*5*	Lab collection (Harris *et al.*, 1997a; CHAPTER 4)
455	W3110	IN (*rrnD-rrnE*)*1*	CGSC 4474
457	SMR457	*dnaA*(Ts) *tnaA300*::Tn*10*	Lab collection
471	JC10287	AB1157 *Δ(srlR-recA)304*	A.J. Clark *via* S.T. Lovett, Brandeis University
503	P90C	*ara Δ(lac-proAB)*XIII *thi*	P.L. Foster, Boston University (Cairns and Foster, 1991)
504	FC29	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F'*proAB*$^+$ *Δlac*]	"
505	FC36	*ara Δ(lac-proAB)*XIII *thi* Rif^r	"
506	FC40	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ*]	"
507	FC82	*ara Δ(lac-proAB)*XIII *thi* *trpE9777* [F'*proAB*$^+$ *lacI33ΩlacZ*]	"
508	FC203	FC40 *recA430*	P.L. Foster, Boston University (Cairns and Foster, 1991; Harris *et al.*, 1994; CHAPTER 2)
532	SMR532	FC40 *dnaB*(Ts) *malB*::Tn*9*	Lab collection; FC40 is SMR506; **NOTE:** may contain a suppressor mutation as this stock produces large and small colonies - see SMR1310 for small colony isolate
533	SMR533	FC40 *malB*::Tn*9*	Lab collection

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
540	SL540	FC40 *dnaE486*(Ts) *zae*::Tn*10d*-Cam	"
575	SZ575	AB1157 *Δ(srlR-recA)304* [pTK2TK1-8]	Lab collection
576	SZ576	AB1157 *Δ(srlR-recA)304* [pAL5]	"
577	HR577	*recD6001::Tn10kan*	Lab collection (Harris *et al.*, 1994; CHAPTER 2)
580	SMR580	FC40 *recB21 argA*::Tn*10*	Lab collection
582	SMR582	FC40 *recD1903*::Tn*10*	"
583	SMR583	FC40 *recJ284*::Tn*10*	Lab collection; **NOTE:** this isolate is mixed - see SMR690 for a *bona fide* FC40 *recJ284*::Tn*10*
589	SMR589	FC40 *recQ61*::Tn*3*	Lab collection (Harris *et al.*, 1994; CHAPTER 2)
593	SMR593	FC40 *recB21*	Lab collection (Harris *et al.*, 1994; CHAPTER 2)
598	SMR598	FC40 *recB270*(Ts)	Lab collection
600	RDK2655	*recG258*::Tn*10miniKan*	R.G. Lloyd *via* R.D. Kolodner, Dana-Farber Cancer Institute, Boston (Lloyd and Buckman, 1991)
601	RDK2615	*ruvC53 eda-51::Tn10*	"
602	RDK2644	*ruvA200 eda-51::Tn10*	N. Sargentini *via* R.G. Lloyd *via* R.D. Kolodner, Dana-Farber Cancer Institute, Boston
603	RDK2641	*ruvA59*::Tn*10*	F. Stahl *via* R.D. Kolodner, Dana-Farber Cancer Institute, Boston

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
605	RDK1873	*ruvB9 zea-3*::Tn*10*	R.D. Kolodner, Dana-Farber Cancer Institute, Boston
620	SZ620	FC40 *mutL211*::Tn*5* **NOTE:** see SMR3404 (Harris *et al.*, 1997c) or SMR3428 (Harris *et al.*, 1997a) for a *bona fide* FC40 *mutL211*::Tn*5*	**NOTE:** this strain (Longerich *et al.*, 1995) probably contains a suppressor of the mutator phenoype as it is not mutator, but the mutator phenotype is 100% transducible with Kan^r
621	SZ621	FC40 *mutU*::Tn*5*	Lab collection (Longerich *et al.*, 1995)
622	SZ622	FC40 *mutS201*::Tn*5* **NOTE:** see SMR3406 (Harris *et al.*, 1997a) for a *bona fide* FC40 *mutS201*::Tn*5*	**NOTE:** this strain (Longerich *et al.*, 1995) is mutator, but the mutator phenotype IS NOT transducible with Kan^r; perhaps the Tn has moved?
623	SZ623	FC40 *mutH471*::Tn*5*	Lab collection (Longerich *et al.*, 1995)
624	SMR624	FC40 *Δ(recA-srlR)306::Tn10*	Lab collection (Harris *et al.*, 1994; CHAPTER 2)
666	RSH1	FC40 *recB270*(Ts) *recJ284*::Tn*10*	SMR598 x P1(SMR583); Tet^r; UV^s at 42°C
682	RSH2	JC9387 *recF*::Tn*3*	SMR121 x P1(SMR686); Amp^r; extremely UV^s
683	RSH16	*trpA36 glyU*(Sup) $glyS_L$ *xyl tsx tyrT*(Sup) *zaj-3053*::Tn*10* *ΔlacX74*	SMR695 x P1(SMR696); Tet^r; Lac$^-$, Pro$^+$, T7^s
684	RSH17	*trpA36 glyU*(Sup) $glyS_L$ *xyl tsx tyrT*(Sup) *zaj-3053*::Tn*10* *ΔlacX74* [F'*proAB*$^+$ *lacI33*Ω*lacZ*]	Mated SMR683 x SMR506; Tet^r; T7^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
685	ES1481	*lacZ53*(Am) *mutS215*::Tn*10* *thyA36 rha-5 metB1 deoC2* [IN(*rrnD-rrnE*)*1*]?	CGSC 7049; **NOTE:** identical to SMR819 (Siegel *et al.*, 1982)
686	RSH5	FC40 *recF*::Tn*3*	SMR506 x P1(*recF*::Tn*3* from JC10990 which is not in the SMR collection); Amp^r; moderately UV^s; *recF* verified by backcrossing into SMR121 (see SMR682)
687	RSH6	C600 *mutS215*::Tn*10*	SMR127 x P1(SMR685); Tet^r; mutator (Nal^r)
688	RSH7	AB1157 *Δ(srlR-recA)304* [pCAT19]	SMR471 transformed with pCAT19; Cam^r
690	RSH8	FC40 *recJ284*::Tn*10*	SMR506 x P1(SMR124); Tet^r; *recJ* verified by backcrossing into SMR121 (see SMR705) (Harris *et al.*, 1994; CHAPTER 2)
691	RSH9	FC29 *Δ(recA-srlR)306*::Tn*10*	SMR504 x P1(SMR624); Tet^r; extremely UV^s (Rosenberg *et al.*, 1995)
692	RSH10	FC40 *recD6001*::*Tn10kan*	SMR506 x P1(SMR577); Kan^r; UV^r, RecD$^-$; **NOTE:** identical to SMR3071 (except selection for Kan^r was not maintained during construction) [(Harris *et al.*, 1994) & CHAPTER 2]
693	RSH11	FC40 *recB21 recD1903*::Tn*10*	SMR593 x P1(SMR582); Tet^r; moderately UV^s; RecD$^-$
695	CH1504	*trpA36 glyU*(Sup) $glyS_L$ *xyl tsx tyrT*(Sup)	C.W. Hill, Penn State; *rhs* recombination strain (Lin *et al.*, 1984)
696	CH5180	M182 *proC zaj-3053*::Tn*10* *ΔlacX74*	C.W. Hill, Penn State; *ΔlacX74 = ΔlacIOPZYA*

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
697	JC15359	AB1157 *tnaA300*::Tn*10* *recF349*	S. Sandler, Berkeley; *recF349* = partial deletion of *recF*
698	JC7623-11	AB1157 *recB21 recC22 sbcB15 sbcC201* Su$^-$	S. Sandler, Berkeley
699	JC7623-11R	AB1157 *recB21 recC22 sbcB15 sbcC201* Su$^-$ *tnaA300*::Tn*10*	"
700	JC7623-11S	AB1157 *recB21 recC22 sbcB15 sbcC201* Su$^-$ *tnaA300*::Tn*10* *recF349*	"
701	FC231	FC40 *lexA3*	P.L. Foster, Boston University (Cairns and Foster, 1991)
702	FC237	FC40 *recAo281*	P.L. Foster, Boston University (Cairns and Foster, 1991); **NOTE:** supposed to be *lexA3*; but is *lexA*$^+$(see SMR898; Appendix II; Foster *et al.*, 1996)
704	FC236	FC40 *recAo281*	P.L. Foster, Boston Uviversity (Cairns and Foster, 1991)
705	RSH12	JC9387 *recJ284*::Tn*10*	SMR121 x P1(SMR690); Tet^r; extremely UV^s
709	RSH13	FC40 *recD6001*::*Tn10kan* *Δ(recA-srlR)306*::Tn*10*	SMR692 x P1(SMR624); Tet^r; extremely UV^s (Harris *et al.*, 1994; CHAPTER 2)
721	RSH14	JC9387 *recQ61*::Tn*3*	SMR121 x P1(SMR589); Amp^r; moderately UV^s; very sick, may contain a growth-defect suppressor

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
722	RSH15	FC40 *recAo281 recF*::Tn*3*	SMR704 x P1(SMR686); Amp^r; moderately UV^s; **NOTE:** identical to SMR1493 (different P1 recipient)
730	RSH18	*trpA36 glyU*(Sup) *glyS*$_L$ *xyl tsx tyrT*(Sup) *zaj-3053*::Tn*10* *ΔlacX74* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ*]	Spontaneous Rif^r isolate of SMR684
731	AM207	*recR252*::Tn*10-9*	R.G. Lloyd *via* R.D. Kolodner, Dana-Farber Cancer Institute; Tn*10-9* confers Kan^r
732	RDK1540	*recN1502*::Tn*5*	R.D. Kolodner, Dana-Farber Cancer Institute
733	RDK1541	*recO1504*::Tn*5*	"
735	RSH19	AB1157 *Δ(srlR-recA)304* [pRH1]	SMR471 transformed with pRH1; Cam^r
736	RSH20	AB1157 *Δ(srlR-recA)304* [pRH2]	SMR471 transformed with pRH2; Cam^r
738	RSH21	FC40 *recB21 recD6001::Tn10kan*	SMR593 x P1(SMR577); Kan^r; moderately UV^s; RecD$^-$ (Harris *et al.*, 1994; CHAPTER 2)
739	RSH22	FC40 [pRH1]	SMR506 transformed with pRH1; Cam^r
740	RSH23	FC40 [pRH2]	SMR506 transformed with pRH2; Cam^r
741	RSH24	C600 [pRH1]	SMR127 transformed with pRH1; Cam^r
742	RSH25	C600 [pRH2]	SMR127 transformed with pRH2; Cam^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
743	RSH26	C600 *mutS215*::Tn*10* [pRH1]	SMR687 transformed with pRH1; Cam^{r}
744	RSH27	C600 *mutS215*::Tn*10* [pRH2]	SMR687 transformed with pRH2; Cam^{r}
745	RSH28	FC40 *recF*::Tn*3* *recA803* *srl-300*::Tn*10*	SMR686 x P1(SMR272); Tet^{r}; more UV^{r} than *recF* parent
746	RSH29	FC40 *recO1504*::Tn*5*	SMR506 x P1(SMR733); Kan^{r}; moderately UV^{s}
747	RSH30	FC40 *recR252*::Tn*10-9*	SMR506 x P1(SMR731); Kan^{r}; moderately UV^{s}
748	RSH31	FC40 *recN1502*::Tn*5*	SMR506 x P1(SMR732); Kan^{r}; moderately UV^{s}
749	RSH32	FC40 *mutS215*::Tn*10*	SMR506 x P1(SMR685); Tet^{r}; mutator (Nal^{r})
750	RSH33	FC40 *recA803 srl-300*::Tn*10*	SMR506 x P1(SMR272); Tet^{r}
752	AB1157	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	S.T. Lovett, Brandeis University (Bachmann, 1996)
758	STL1605	AB1157 *Δ(xseA-guaB) zff-3139*::Tn*10kan*	S.T. Lovett, Brandeis University (Vales *et al.*, 1979)
762	RSH35	FC40 *recAo281 srl-300*::Tn*10* *recF*::Tn*3*	SMR722 x P1(SMR235); Tet^{r}; UV^{r} at 42°C
769	RSH36	FC29 *trp*::Tn*5*	SMR504 x P1(SMR417); Kan^{r}; Trp^{-}
785	CJ300	*Δ(gal-bio) thi-1 relA1 spoT1 ΔpolA*::*kan* [F$'$ *polA*$^{+}$ Cam^{r}]	C. Joyce, Yale University (Joyce and Grindley, 1984)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
788	RSH37	FC40 *recG258*::Tn*10miniKan* [this strain may contain a suppressor of *recG* because (i) it is UV^r and should be slightly UV^s, and (ii) its adaptive mutation phenotype is variable; however, the *recG* mutation and associated phenotypes (e.g. UV^s) are cotransducible.	SMR506 x P1(SMR600); Kan^r; NOT UV^s; NOTE: SLAM phenotype of this strain is variable - see SMR1982 for a *bona fide* FC40 *recG* strain (Harris *et al.*, 1996)
789	RSH38	FC40 *ruvC53 eda-51*::*Tn10*	SMR506 x P1(SMR601); Tet^r; UV^s; (Harris *et al.*, 1996; CHAPTER 3)
790	RSH39	W3110 *trpC9941* Rif^r	Spontaneous Rif^r isolate of SMR147
792	RSH40	FC40 *recD6001*::*Tn10kan* *mutS215*::Tn*10*	SMR692 x P1(SMR685); Tet^r; mutator (Nal^r)
795	RSH41	FC82 *Δ(recA-srlR)306*::Tn*10*	SMR507 x P1(SMR624); Tet^r; extremely UV^s
796	RSH42	FC82 *recJ284*::Tn*10*	SMR507 x P1(SMR690); Tet^r; *recJ* verified by backcrossing into SMR121
797	RSH43	FC82 *recQ61*::Tn*3*	SMR507 x P1(SMR589); Amp^r; *recQ* verified by backcrossing into SMR121
798	RSH44	FC82 *recD6001*::*Tn10kan*	SMR507 x P1(SMR577); Kan^r; RecD$^-$
799	RSH45	FC40 *ruvC53 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR789 x P1(SMR788); Kan^r, extremely UV^s; NOTE: identical to SMR2040 & SMR2041 (different P1 donors) (Harris *et al.*, 1996; CHAPTER 3)
800	RSH46	W3110 *zaj-3053*::Tn*10* *ΔlacX74*	SMR455 x P1(SMR696); Tet^r; Lac$^-$, Pro$^+$
816	MG1655	"Wildtype"	CGSC 6300

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
817	DM49	AB1157 *lexA3*	CGSC 6367 (Mount *et al.*, 1972)
818	MV1138	AB1157 *rfa-300 recAo281 srl-300*::Tn*10*	CGSC 6706; **NOTE:** (i) *recAo281* was transduced in from *E.coli* B (ii) genotype slightly different from SMR853 & SMR858 (but supposed to be identical) (Volkert *et al.*, 1981)
819	ES1481	*lacZ53*(Am) *mutS215*::Tn*10* *thyA36 rha-5 metB1 deoC2* [IN(*rrnD-rrnE*)*1*]?	CGSC 7049; **NOTE:** identical to SMR685 (Siegel *et al.*, 1982)
820	RSH47	FC40 *lexA3 malB*::Tn*9*	SMR701 x P1(SMR533); Cam^r; Mal$^-$, UV^s
821	RSH48	AB1157 *lexA3 malB*::Tn*9*	SMR817 x P1(SMR533); Cam^r; Mal$^-$, UV^s
822	RSH49	AB1157 *malB*::Tn*9*	SMR817 x P1(SMR533); Cam^r; Mal$^-$, UV^r
823 to 826	CW1 to CW4	FC40 *mutS201*::Tn*5* Lac$^+$	Spontaneous Lac$^+$ isolates 1-4 of SMR622; **NOTE:** see SMR622
827	RSH50	W3110 *zaj-3053*::Tn*10*	SMR455 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
828	RSH51	P90C *zaj-3053*::Tn*10*	SMR503 x P1(SMR827); Tet^r; Pro$^-$, Lac$^-$
829	RSH52	C600 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10*	SMR127 x P1(SMR828); Tet^r; Pro$^-$
830	RSH53	C600 *zaj-3053*::Tn*10*	SMR127 x P1(SMR828); Tet^r; Pro$^+$
831	RSH54	MG1655 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10*	SMR816 x P1(SMR828); Tet^r; Pro$^-$, Lac$^-$
832	RSH55	MG1655 *zaj-3053*::Tn*10*	SMR816 x P1(SMR828); Tet^r; Pro$^+$, Lac$^+$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
833	RSH56	W3110 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10*	SMR455 x P1(SMR828); Tet^r; Pro$^-$, Lac$^-$
834	CW5	FC29 *tnaA300*::Tn*10*	SMR504 x P1(SMR457); Tet^r; Ts$^+$
835	RSH57	FC40 *recA200*(Ts) *srl-300*::Tn*10*	SMR506 x P1(SMR235); Tet^r; extremely UV^s at 42°C
836	DM1180	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 tsx-33*(?) *supE44 galK2 sulA211 hisG4 rfbD1 mgl-51 recA441 rpsL31 kdgK51 xyl-5 mtl-1 lexA3*	CGSC 6550 (Mount, 1977)
837	DM1187	*thr-1 leuB6 Δ(gpt-proA)62 sulA211 hisG4 recA441 thi-1*(?) *lexA3 lexA51 ara-14 galK2 xyl-5 mtl-1 rpsL31 tsx-33*(?) *supE44*	CGSC 6551 (Mount, 1977)
838	HR838	JC11450 *ΔxonA300*::*CAT his*$^+$	Lab collection (Harris *et al.*, 1997c; Razavy *et al.*, 1996; APPENDIX IV)
839	HR839	JC11450 *ΔxonA300*::*CAT his*$^-$	Lab collection
840	PN103	K91 *priA2*::*kan*	S. T. Lovett, Brandeis University; *priA2*::*kan* is a simple insertion mutation (Nurse *et al.*, 1991); **NOTE:** was purified on and frozen to the collection in rich media; *priA* strains are LBH^s, therefore, this strain probably contains a growth-defect suppressor
841	RSH58	FC40 *lexA3 malB*::Tn*9*	SMR506 x P1(SMR821); Cam^r; Mal$^-$, moderately UV^s
843	SL843	FC40 *dam13*::Tn*9*	Lab collection (Longerich *et al.*, 1995)
844	RSH59	FC40 *recA200*(Ts)	SMR835 x P1(SMR506); Srl$^+$ at 32°C; UV^r at 42°C

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
845	RSH60	FC40 *lexA3 malB*::Tn9 *recAo281 srl-300*::Tn*10*	SMR841 x P1(SMR818); Tet^r; more UV^r than *lexA3* but slightly less than *lex*$^+$
846	RSH61	FC40 *recAo281 srl-300*::Tn*10*	SMR506 x P1(SMR818); Tet^r; see SMR866 for confirmation of *recAo281*
851	RSH62	FC40 *lexA3 malB*::Tn9 *recF*::Tn*3*	SMR841 x P1(SMR686); Amp^r; UV^s similar to *lexA3* & more sensitive than *recF*
853	MV1138	*recAo281 srl-300*::Tn*10 thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	M. Volkert, University of Massachusetts; **NOTE:** (i) *recAo281* was transduced in from *E.coli* B (ii) genotype slightly different than SMR818 & SMR858 (but supposed to be identical) (Volkert *et al.*, 1981)
854	JC11867	*lexA3 recAo281 srl-300*::Tn*10 thr-1 leu-6 ara-14 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	A. J. Clark *via* M. Volkert, University of Massachusetts; **NOTE:** the genotype is slightly different than JC11867 provided from A.J. Clark (SMR859) (Volkert *et al.*, 1981)
855	RSH63	FC40 *lexA3 recAo281 srl-300*::Tn*10*	SMR701 x P1(SMR762); Tet^r; UV^r relative to *lexA3*; **NOTE:** identical to SMR876 (different parents)
856	RSH64	FC40 *lexA3 recAo281*	SMR855 x P1(SMR704); Srl$^+$; Tet^s, UV^r relative to *lexA3*; **NOTE:** identical to SMR877 (different parents)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
858	MV1138	*recAo281 srl-300*::Tn*10 thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	A. J. Clark, Berkeley; **NOTE:** (i) *recAo281* was transduced in from *E.coli* B (ii) genotype slightly different than SMR818 & SMR853 (but supposed to be identical) (Volkert *et al.*, 1981)
859	JC11867	*lexA3 recAo281 srl-300*::Tn*10 thr-1 leu-6* [*recB21 recC22*(?)] *ara-14 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	A. J. Clark, Berkeley; **NOTE:** the genotype is slightly different than JC11867 provided by M. Volkert (SMR854) (Volkert *et al.*, 1981)
860	CAG 12131	MG1655 *zac-3093*::Tn*10kan*	C. Gross, UCSF; Tn at 2' linked to *polB* (Singer *et al.*, 1989)
861	CAG 18436	MG1655 *zae-502*::Tn*10*	C. Gross, UCSF; Tn at 4.75' linked to *dnaE* and *dnaQ* (Singer *et al.*, 1989)
862	CAG 12080	MG1655 *zah-281*::Tn*10*	C. Gross, UCSF; Tn at 7.75' linked to *lac* (Singer *et al.*, 1989)
863	CAG 18413	MG1655 *tsx-3100*::Tn*10kan*	C. Gross, UCSF; Tn at 9.5' linked to *lac* (Singer *et al.*, 1989)
864	CAG 18558	MG1655 *zid-3162*::Tn*10kan*	C. Gross, UCSF; Tn at 83' linked to *recF* (Singer *et al.*, 1989)
865	CAG 18609	MG1655 *malF3180*::Tn*10kan*	C. Gross, UCSF; Tn at 91.5' linked to *lexA* (Singer *et al.*, 1989)
866	RSH65	FC40 *lexA3 malB*::Tn*9 recAo281 srl-300*::Tn*10*	SMR841 x P1(SMR846); Tet^r; more UV^r than *lexA3* but slightly less than *lex*$^+$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
867	RSH66	FC40 *recAo281 lexA3 malB*::Tn*9*	SMR704 x P1(SMR820); Cam^{r}; Mal^{-}, more UV^{r} than *lexA3* but slightly less than *lex*$^{+}$; *lexA3* confirmed by backcross into SMR506
868	RSH67	FC40 *lexA3*	SMR841 x P1(SMR506); Mal^{+}; Cam^{s}, UV^{s}
869	SH2101	*leu ara* Δ(*pro-lac*) *thi* Sm^{r} *polB*Δ*1*::ΩSm-Sp (small-size colony)	M. Goodman, University of Southern California; streaked to LBH and displayed small, medium and large colony morphologies (Escarcellar *et al*., 1994)
870	SH2101	*leu ara* Δ(*pro-lac*) *thi* Sm^{r} *polB*Δ*1*::ΩSm-Sp (medium-size colony)	"
871	SH2101	*leu ara* Δ(*pro-lac*) *thi* Sm^{r} *polB*Δ*1*::ΩSm-Sp (large-size colony)	"
872	RSH68	C600 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^{r}	Spontaneous Rif^{r} isolate of SMR829
873	RSH69	MG1655 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^{r}	Spontaneous Rif^{r} isolate of SMR831
874	RSH70	W3110 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^{r}	Spontaneous Rif^{r} isolate of SMR833
875	RSH71	C600 *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^{r} [F'*proAB*$^{+}$ *lacI33ΩlacZ*]	Mated SMR872 x SMR506; Pro^{+} & Tet^{r}; R17^{s}
876	RSH72	FC40 *lexA3 recAo281 srl-300*::Tn*10*	SMR868 x P1(SMR853); Tet^{r}; more UV^{r} than *lexA3* but slightly less than *lex*$^{+}$; NOTE: identical to SMR855 (different parents)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
877	RSH73	FC40 *recAo281 lexA3*	SMR867 x P1(SMR701); Mal$^+$, Cam^s; UV^r; **NOTE:** identical to SMR856 (different parents)
878	GW2707	*thr-1 ara-14*(?) *leuB6 Δ(argF-lac)169 tsx-33*(?) *supE44*(?) *galK2 sulA211 hisG4 recA441 rpsL31*(?) *xyl-5*(?) *ilv*(Ts) *argE3 thi-1*(?) *dinD1*::Mu *d*(Ap *lac*) *lexA71*::Tn*5* <[pGW600]>	G. Walker, MIT; **NOTE:** this strain has lost pGW600 (B. Bachmann, personal communication) (Kruger *et al.*, 1983)
879	DM5007	*thyA36 deoC2 rha-5 lacZ53 strA51 malB45 zja-505*::Tn*10*	D. Mount *via* M. Volkert, University of Massachusetts; Tn at 90' is linked to *lexA*
880	MV1159	*lexA3 recAo281 thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	M. Volkert, University of Massachusetts (Volkert *et al.*, 1981)
881	RSH74	*leu ara* Δ(*pro-lac*) *thi* Sm^r *polBΔ1*::ΩSm-Sp *zac-3093*::Tn*10kan*	SMR869 x P1(SMR860); Kan^r; Spc^r, Leu$^-$
882	RSH75	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ zaj-3053*::Tn*10*]	SMR506 x P1(SMR696); Tet^r; see SMR884 for a verification of the episomal location of the Tn; **NOTE:** identical to SMR883 (constructed independently)
883	RSH76	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ zaj-3053*::Tn*10*]	SMR506 x P1(SMR696); Tet^r; see SMR885 for a verification of the episomal location of the Tn; **NOTE:** identical to SMR882 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
884	RSH77	*mutU*::Tn*5* Str^r [F′*proAB*$^+$ Lac$^+$ revertant of *lacI33ΩlacZ* *zaj-3053*::Tn*10*]	Mated SMR362 x spontaneous Lac$^+$ isolate of SMR882; selected Kan^r & Tet^r & Lac$^+$; T7^r, mutator (Nal^r); **NOTE:** phenotypically identical to SMR885 (different F donor)
885	RSH78	*mutU*::Tn*5* Str^r [F′*proAB*$^+$ Lac$^+$ revertant of *lacI33ΩlacZ* *zaj-3053*::Tn*10*]	Mated SMR362 x spontaneous Lac$^+$ isolate of SMR883; selected Kan^r & Tet^r & Lac$^+$; T7^r, mutator (Nal^r); **NOTE:** phenotypically identical to SMR884 (different F donor)
886	RSH79	W3110 *trpC9941 lacI33ΩlacZ* *zaj-3053*::Tn*10*	SMR147 x P1(SMR882); Tet^r; Lac$^-$; **NOTE:** (i) lac frameshift mutation reverts at a very low frequency in the chromosome; (ii) DNA sequencing confirmed *lacI33-lacZ* fusion gene; (iii) P1 transduction confirmed linkage between the Tn and *lac*; (iv) identical to SMR887 (different P1 donor)
887	RSH80	W3110 *trpC9941 lacI33ΩlacZ* *zaj-3053*::Tn*10*	SMR147 x P1(SMR883); Tet^r; Lac$^-$; **NOTE:** (i) see SMR886; (ii) identical to SMR886 (different P1 donor)
888	RSH81	W3110 *trpC9941 lacI33ΩlacZ* *zaj-3053*::Tn*10* Rif^r	Spontaneous Rif^r isolate of SMR886; **NOTE:** (i) see SMR886; (ii) identical to SMR889 (different parent)
889	RSH82	W3110 *trpC9941 lacI33ΩlacZ* *zaj-3053*::Tn*10* Rif^r	Spontaneous Rif^r isolate of SMR887; **NOTE:** (i) see SMR886; (ii) identical to SMR888 (different parent)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
890	RSH83	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F′*lacI33ΩlacZ zaj-3053*::Tn*10*]	SMR506 x P1(SMR800); Tet^r; Lac$^-$ (revertable), confirmed episomal location of the Tn*10* by mating F′ into SMR74 (see SMR891)
891	RSH84	C600 *dam13*::Tn*9* [F′*lacI33ΩlacZ zaj-3053*::Tn*10*]	Mated SMR74 + SMR890; Cam^r & Tet^r
892	RSH85	W3110 *ΔpolA*::*kan*	SMR455 x P1(SMR785); Kan^r; LBH^s
893	RSH86	C600 *ΔpolA*::*kan*	SMR127 x P1(SMR785); Kan^r; LBH^s
894	RSH87	W3110 *ΔlacX74* *zaj-3053*::Tn*10*	SMR455 x P1(SMR696); Tet^r; Pro$^+$, Lac$^-$
895	RSH88	W3110 *ΔlacX74* *zaj-3053*::Tn*10* Rif^r	Spontaneous Rif^r isolate of SMR894
896	RSH89	C600 *ΔlacX74* (or *lacY1*?) *zaj-3053*::Tn*10* Rif^r	SMR127 x P1(SMR696); Tet^r; Pro$^+$; spontaneously Rif^r
897	RSH90	FC40 *recA200*(Ts) *srl-300*::Tn*10*	SMR702 x P1(SMR235); Tet^r; UV^r at 30°C & extremely UV^s at 42°C; **NOTE:** this strain is supposed to be *lexA3*, but is apparently not, because it is UV^r at 30°C (see SMR898 & APPENDIX II)
898	RSH91	FC40 *recAo281 srl-300*::Tn*10*	SMR702 x P1(SMR235); Tet^r; UV^r at 30°C & 42°C; **NOTE:** this strain is supposed to be *lexA3*, but is apparently not, because it is UV^r (see SMR897 and APPENDIX II)
899	RSH92	FC40 *lexA3 recA200*(Ts) *srl-300*::Tn*10*	SMR701 x P1(SMR235); Tet^r; extremely UV^s at 42°C

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1013	SMR1013	FC40 Lac$^+$	Lab collection
1167 to 1190	SMR1167 to SMR1190	FC40 Lac$^+$	Lab collection (Longerich, 1997)
1231 to 1251	SMR1231 to SMR1251	FC40 *recD6001*::Tn*10kan* Lac$^+$	Lab collection (Longerich, 1997)
1252	RSH93	FC40 *recA801 srl-300*::Tn*10*	SMR506 x P1(SMR85); Tet^r
1253	RSH94	FC40 *recA801 srl-300*::Tn*10* *recF*::Tn*3*	SMR1252 x P1(SMR686); Amp^r
1254	RSH95	W3110 *trpC9941* *zaj-3053*::Tn*10*	SMR147 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
1255	RSH96	W3110 *trpC9941 ΔlacX74* *zaj-3053*::Tn*10*	SMR147 x P1(SMR696); Tet^r; Pro$^+$, Lac$^-$
1256	RSH97	C600 *zaj-3053*::Tn*10*	SMR127 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
1257	RSH98	FC40 *recAo281 recF*::Tn*3*	SMR702 x P1(SMR686); Amp^r; slightly more UV^r than *recF*
1258	RSH99	FC40 *recAo281 srl-300*::Tn*10*	SMR702 x P1(SMR762); Tet^r; UV^r like SMR506
1267	RSH100	C600 *thr*$^+$ *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR875 x P1(SMR455); Thr$^+$; Leu$^-$
1268	RSH101	C600 *thr*$^+$	SMR127 x P1(SMR455); Thr$^+$; Leu$^-$
1269 to 1272	CW6 to CW9	FC40 *mutL211*::Tn*5* Lac$^+$	Spontaneous Lac$^+$ isolates 1-4 of SMR620; **NOTE:** see SMR620
1301	RSH102	FC40 *recAo281*	SMR846 x P1(SMR880); Srl$^+$; Tet^s

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1302	RSH103	FC40 *lexA3 recAo281*	SMR876 x P1(SMR880); Srl$^+$; Tet^s, UV^r *vs lexA3*
1303	LE30	F$^-$ *mutD5 rpsL galU95*	R. M. Schaaper *via* L. Reha-Kranz, University of Alberta
1304	RSH104	C600 *thr*$^+$ *leu*$^+$	SMR1268 x P1(SMR455); Leu$^+$
1305	RSH105	C600 *thr*$^+$ *leu*$^+$ *Δ(lac-proAB)*XIII *zaj-3053*::Tn*10* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1267 x P1(SMR455); Leu$^+$
1306	RM3980	MG1655 *ΔdnaQ903::tet spq-2*	R. Maurer, Case Western Reserve University (Slater *et al.*, 1994)
1307	RM4196	MG1655 *zae-3095*::Tn*10kan spq-2*	R. Maurer, Case Western Reserve University; **NOTE:** Tn at 4.6' linked to *dnaQ* (not to *spq-2*) (Slater *et al.*, 1994)
1308	RM4193	MG1655 *holE202::cat*	R. Maurer, Case Western Reserve University (Slater *et al.*, 1994)
1309	RM4848	AB1157 *recA430 srl-300*::Tn*10* *zjg-2086*::Tn*10kan* *holC102::cat*	R. Maurer, Case Western Reserve University
1310	RSH106	FC40 *dnaB*(Ts) *malB*::Tn*9*	Small colony isolate of SMR532
1312	RSH107	M182 *proC zaj-3053*::Tn*10* *ΔlacX74* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated SMR696 x SMR506; Tet^r & T7^r; Pro$^-$
1313	RSH108	M182 *proC zaj-3053*::Tn*10* *ΔlacX74* [F'*proAB*$^+$ *lacI33ΩlacZ*$^+$]	Mated SMR696 x SMR1013; Tet^r & Lac$^+$; R17^s, but not as sensitive as SMR1013

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1314	RSH109	M182 *zaj-3053*::Tn*10* *ΔlacX74*	SMR696 x P1(SMR455); Pro$^+$; Lac$^-$, Tet^r
1315	RSH110	FC40 *holE202*::*cat*	SMR506 x P1(SMR1308); Cam^r
1316	RSH111	MG1655 Rif^r	Spontaneous Rif^r isolate of SMR816
1326	RSH112	F$^-$ *mutD5 rpsL galU95 zae-502*::Tn*10*	SMR1303 x P1(SMR861); Tet^r; mutator (Nal^r & Rif^r); Tn is linked to *mutD5*
1327	RSH113	W3110 Rif^r	Spontaneous Rif^r isolate of SMR455
1356	GC2641 [pW18]	594 *sulA85 lac gal* [pW18]	R. Fuchs *via* G. Maenhaut-Michel, Belgium
1357	GC2641 [pW17]	594 *sulA85 lac gal* [pW17]	"
1358	GC2641 [pX2]	594 *sulA85 lac gal* [pX2]	"
1359	RSH114	M182 *ΔlacX74*	SMR696 x P1(SMR455); Pro$^+$; Lac$^-$, Tet^s
1360	RSH115	MG1655 *zae-3095*::Tn*10Kan* *spq-2 zae*::Tn*10d*-Cam	SMR1307 x P1[*dnaE486*(Ts) *zae*::Tn*10d*-Cam from NR9779 (R. Schaaper, NIEHS), which is not in the SMR collection]; Cam^r; Ts$^+$; Tn*10d-Cam* is linked to *spq-2*, an allele of *dnaE*
1398	RSH116	FC40 *mutD5 zae-502*::Tn*10*	SMR506 x P1(SMR1326); Tet^r; strong mutator (Nal^r)
1399	RSH117	FC40 *zae-502*::Tn*10*	SMR506 x P1(SMR1326); Tet^r; non-mutator (Nal^r)
1400	RSH118	FC40 [pW18]	SMR506 transformed with pW18; Amp^r; Tet^s (Torkelson *et al.*, 1997)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1401	RSH119	FC40 [pW17]	SMR506 transformed with pW17; Amp^r; Tet^s (Torkelson *et al.*, 1997)
1402	RSH120	FC40 [pX2]	SMR506 transformed with pX2; Amp^r; Tet^s (Torkelson *et al.*, 1997)
1403	SZ1403	JC11450 *ΔxonA300*::*CAT recJ284*::Tn*10*	Lab collection (Harris *et al.*, 1997c; Razavy *et al.*, 1996; APPENDIX IV)
1405	RSH121	M182 *ΔlacX74 malB*::Tn*9*	SMR1359 x P1(SMR533); Cam^r; Lac$^-$, Mal$^-$
1406	RSH122	M182 *ΔlacX74 malB*::Tn*9* Rif^r	Spontaneous Rif^r isolate of SMR1405
1407	RSH123	C600 *thr*$^+$ *leu*$^+$ Rif^r	Spontaneous Rif^r isolate of SMR1304
1463	RSH124	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F' *proAB*$^+$ *lac*$^+$ *zah-281*::Tn*10*]	SMR506 x P1(SMR862); Tet^r; Lac$^+$; Tn on F' confirmed by mating into SMR1406 (see SMR1466); **NOTE:** identical to SMR1464 (constructed independently)
1464	RSH125	*ara Δ(lac-proAB)*XIII *thi* Rif^r [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR506 x P1(SMR862); Tet^r; Lac$^-$; Tn on F' confirmed by mating into SMR1406 (see SMR1465); **NOTE:** identical to SMR1463 (constructed independently)
1465	RSH127	M182 *ΔlacX74 malB*::Tn*9* Rif^r [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	Mated SMR1406 x SMR1464; Tet^r & Cam^r; Lac$^-$, R17^s; **NOTE:** identical to SMR1466 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1466	RSH126	M182 *ΔlacX74 malB*::Tn9 Rif^{r} [F' *proAB*$^{+}$ *lac*$^{+}$ *zah-281*::Tn*10*]	Mated SMR1406 x SMR1463; Tet^{r} & Cam^{r}; Lac^{-}, R17^{s}; **NOTE:** identical to SMR1465 (constructed independently)
1467	RSH128	*ara Δ(lac-proAB)*XIII *thi* Rif^{r} [F' *proAB*$^{+}$ *lacI33ΩlacZ zah*::Tn*10*::*λTSK*]	SMR1464 lysogenized with λTSK; Kan^{r}; Str^{r}, Tet^{s}; **NOTE:** grow at <32°C
1468	RSH129	C600 *thr*$^{+}$ *leu*$^{+}$ Rif^{r} *malB*::Tn9	SMR1407 x P1(SMR533); Cam^{r}; Mal^{-}
1471	RSH130	*ara Δ(lac-proAB)*XIII *thi* Rif^{r} [F' *proAB*$^{+}$ *lacI33ΩlacZ zah*::Tn*10kan*]	SMR1467 cured of prophage by streaking on LBH Kan and incubating at 42°C; Str^{s}, Tet^{s}
1472	RSH131	M182 *ΔlacX74 malB*::Tn9 Rif^{r} *ΔpolA*::*kan* (?) [F' *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1465 x P1(SMR785); Kan^{r}; **NOTE:** (i) this was the only isolate obtained from 2 transductions, whereas the same lysates produced 20-50 Kan^{r} isolates of SMR696 and SMR127, suggesting that *ΔpolA* is lethal in this strain; (ii) possibly heterozygous for *polA*; (iii) genotypically identical to SMR1661 & 1662 (see APPENDIX III)
1489	RSH132	FC40 *priA2*::*kan*	SMR506 x P1(SMR840); Kan^{r}; LBH^{s}
1490	RSH133	M182 *proC zaj-3053*::Tn*10* *ΔlacX74 ΔpolA*::*kan*	SMR696 x P1(SMR785); Kan^{r}; **NOTE:** LBH^{r} & should be LBH^{s}, therefore it may have a suppressor of *polA*
1491	RSH134	C600 *thr*$^{+}$ *leu*$^{+}$ *lac*$^{+}$ Rif^{r} *malB*::Tn9	SMR1468 x P1(SMR455); Lac^{+}

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1492	RSH135	FC40 *recAo281 srl-300*::Tn*10 recF*::Tn*3*	SMR846 x P1(SMR686); Amp^r; UV^s like *recF*
1493	RSH136	FC40 *recAo281 recF*::Tn*3*	SMR1301 x P1(SMR686); Amp^r; UV^s like *recF*; **NOTE:** identical to SMR722 (different P1 recipient)
1494	RSH137	FC40 *lexA3 malB*::Tn*9 recAo281 srl-300*::Tn*10 recF*::Tn*3*	SMR845 x P1(SMR686); Amp^r; UV^s like *recF*
1496	RSH138	FC40 *recD6001::Tn10kan recF*::Tn*3*	SMR692 x P1(SMR686); Amp^r; UV^s like *recF*; **NOTE:** identical to SMR1497 (constructed differently)
1497	RSH139	FC40 *recF*::Tn*3 recD6001::Tn10kan*	SMR686 x P1(SMR577); Kan^r; UV^s like *recF*; **NOTE:** identical to SMR1496 (constructed differently)
1498	RSH140	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn9 *proC zaj-3053*::Tn*10 ΔlacX74*	SMR1491 x P1(SMR696); Tet^r; Lac$^-$, Pro$^-$
1499	RSH141	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn9 *ΔlacX74*	RSH1498 x P1(SMR455); Pro$^+$; Tet^s, Lac$^-$
1500	RSH142	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn9 *zaj-3053*::Tn*10 ΔlacX74*	RSH1498 x P1(SMR455); Pro$^+$; Tet^r, Lac$^-$
1501	RSH143	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn9 *ΔlacX74* [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	Mated SMR1499 x SMR1464; Tet^r & Cam^r; R17^s; **NOTE:** this strain is Lac$^+$; *supE44*(=SuII$^+$) of C600 may be a suppressor of the *lac* frameshift mutation

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1502	RSH144	FC40 *ΔdnaQ903::tet spq-2 zae*::Tn*10d*-Cam	SMR506 x P1(SMR3640); Cam^{r} & Tet^{r}; **NOTE:** R17^{r} & should be R17^{s}-- see SMR1547 and SMR1548 for transductants with proper phenotypes
1503	RSH145	*ara Δ(lac-proAB)*XIII *thi* Rif^{s} [F*proAB*$^{+}$ *lac*$^{+}$ *zah-281*::Tn*10*]	SMR504 x P1(SMR862); Tet^{r}; Lac^{+}
1504	RSH146	*ara Δ(lac-proAB)*XIII *thi* Rif^{s} [F*proAB*$^{+}$ *Δlac zah-281*::Tn*10*]	SMR504 x P1(SMR862); Tet^{r}; Lac^{-}
1505	RSH147	*ara Δ(lac-proAB)*XIII *thi* Rif^{s} *malB*::Tn*9* [F*proAB*$^{+}$ *Δlac zah-281*::Tn*10*]	SMR1504 x P1(SMR533); Cam^{r}; Mal^{-}
1508	RSH148	*ara Δ(lac-proAB)*XIII *thi* Rif^{s} *malB*::Tn*9 zac-3093*::Tn*10kan* [F*proAB*$^{+}$ *Δlac zah-281*::Tn*10*]	SMR1505 x P1(SMR860); Kan^{r}
1509	RSH149	FC29 *recQ61*::Tn*3*	SMR504 x P1(SMR589); Amp^{r}
1547	RSH150	FC40 *ΔdnaQ903::tet spq-2 zae*::Tn*10d*-Cam	SMR506 x P1(SMR3640); Cam^{r} & Tet^{r}; Lac^{-}, but revertable, R17^{s}; **NOTE:** identical to SMR1548 (constructed independently)
1548	RSH151	FC40 *ΔdnaQ903::tet spq-2 zae*::Tn*10d*-Cam	SMR506 x P1(SMR3640); Cam^{r} & Tet^{r}; Lac^{-}, but revertable, R17^{s}; **NOTE:** identical to SMR1547 (constructed independently)
1549	RSH152	FC40 *ruvA200 eda-51::Tn10*	SMR506 x P1(SMR602); Tet^{r}; moderately UV^{s} (Harris *et al.*, 1996; CHAPTER 3)
1550	RSH153	FC40 *eda-51::Tn10*	SMR506 x P1(SMR602); Tet^{r}; UV^{r}

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1551	RSH154	FC40 *ruvA59*::Tn*10*	SMR506 x P1(SMR603); Tet^r; moderately UV^s; **NOTE:** identical to SMR1983 (constructed independently) (Harris *et al.*, 1996; CHAPTER 3)
1552	RSH155	FC40 *ruvB9 zea-3*::Tn*10*	SMR506 x P1(SMR605); Tet^r; moderately UV^s (Harris *et al.*, 1996; CHAPTER 3)
1553	RSH156	FC40 *zea-3*::Tn*10*	SMR506 x P1(SMR605); Tet^r; UV^r
1560	RSH157	MG1655 Rif^r *malB*::Tn*9*	SMR1316 x P1(SMR533); Cam^r; Mal$^-$
1561	RSH158	W3110 Rif^r *malB*::Tn*9*	SMR1327 x P1(SMR533); Cam^r; Mal$^-$
1563	RSH159	FC40 *ruvA200 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR1549 x P1(SMR788); Kan^r; extremely UV^s; **NOTE:** identical to SMR2034 & SMR2035 (different P1 donors) (Harris *et al.*, 1996; CHAPTER 3)
1564	RSH160	FC40 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan*	SMR1551 x P1(SMR788); Kan^r; extremely UV^s; **NOTE:** identical to 1984 (different parents), SMR2036 & SMR2037 (different P1 donors) (Harris *et al.*, 1996; CHAPTER 3)
1565	RSH161	FC40 *ruvB9 zea-3*::Tn*10* *recG258*::Tn*10miniKan*	SMR1552 x P1(SMR788); Kan^r; extremely UV^s; **NOTE:** identical to SMR2038 & SMR2039 (different P1 donors) (Harris *et al.*, 1996; CHAPTER 3)
1594	RSH162	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *proC zaj-3053*::Tn*10* *ΔlacX74*	SMR1491 x P1(SMR696); Tet^r; Pro$^-$, Lac$^-$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1595	RSH163	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1491 x P1(SMR696); Tet^r; Pro$^+$, Lac$^-$
1596	RSH164	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *proC* *zaj-3053*::Tn*10*	SMR1491 x P1(SMR696); Tet^r; Pro$^-$, Lac$^+$
1597	RSH165	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10*	SMR1491 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
1598	RSH166	MG1655 Rif^r *malB*::Tn*9* *proC* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1560 x P1(SMR696); Tet^r; Pro$^-$, Lac$^-$
1599	RSH167	MG1655 Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1560 x P1(SMR696); Tet^r; Pro$^+$, Lac$^-$
1600	RSH168	MG1655 Rif^r *malB*::Tn*9* *proC* *zaj-3053*::Tn*10*	SMR1560 x P1(SMR696); Tet^r; Pro$^-$, Lac$^+$
1601	RSH169	MG1655 Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10*	SMR1560 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
1602	RSH170	W3110 Rif^r *malB*::Tn*9* *proC* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1561 x P1(SMR696); Tet^r; Pro$^-$, Lac$^-$
1603	RSH171	W3110 Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1561 x P1(SMR696); Tet^r; Pro$^+$, Lac$^-$
1604	RSH172	W3110 Rif^r *malB*::Tn*9* *proC* *zaj-3053*::Tn*10*	SMR1561 x P1(SMR696); Tet^r; Pro$^-$, Lac$^+$
1605	RSH173	W3110 Rif^r *malB*::Tn*9* *zaj-3053*::Tn*10*	SMR1561 x P1(SMR696); Tet^r; Pro$^+$, Lac$^+$
1606	RSH174	JC9387 Rif^r	Spontaneous Rif^r isolate of SMR121
1607	RSH175	JC11450 Rif^r	Spontaneous Rif^r isolate of SMR122
1608	RSH176	JC9388 Rif^r	Spontaneous Rif^r isolate of SMR165
1609	RSH177	FC40 *ruvA*::Tn*10*::λTSK	SMR1551 lysogenized with λTSK; Kan^r; Str^r, Tet^s; NOTE: grow at <32°C

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1610	RSH178	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *ΔlacX74*	SMR1594 x P1(SMR506); Pro$^+$; Tet^s, Lac$^-$
1611	RSH179	MG1655 Rif^r *malB*::Tn*9* *ΔlacX74*	SMR1598 x P1(SMR506); Pro$^+$; Tet^s, Lac$^-$
1612	RSH180	W3110 Rif^r *malB*::Tn*9* *ΔlacX74*	SMR1602 x P1(SMR506); Pro$^+$; Tet^s, Lac$^-$
1618	RSH181	JC9387 Rif^r *leu*$^+$	SMR1606 x P1(SMR816); Leu$^+$
1619	RSH182	JC11450 Rif^r *leu*$^+$	SMR1607 x P1(SMR816); Leu$^+$
1620	RSH183	JC9388 Rif^r *leu*$^+$	SMR1608 x P1(SMR816); Leu$^+$
1621	RSH184	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *ΔlacX74* [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	Mated SMR1610 x SMR1464; Tet^r & Cam^r; R17^s; NOTE: leaky Lac$^+$ phenotype- this may be due to the suppressor, *supE44* (=SuII$^+$), in the C600 background because the strain is genotypically Lac$^-$
1622	RSH185	MG1655 Rif^r *malB*::Tn*9* *ΔlacX74* [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	Mated SMR1611 x SMR1464; Tet^r & Cam^r; R17^s; Lac$^-$ (revertable)
1623	RSH186	W3110 Rif^r *malB*::Tn*9* *ΔlacX74* [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	Mated SMR1612 x SMR1464; Tet^r & Cam^r; R17^s; Lac$^-$ (revertable)
1624	RSH187	FC40 *ruvA59*::Tn*10kan*	SMR1609 cured of prophage by streaking on LBH Kan at 43°C; Str^s, Tet^s, moderately UV^s
1627	RSH188	MG1655 Str^r	Spontaneous Str^r isolate of SMR816

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1628	RSH189	W3110 Str^{r}	Spontaneous Str^{r} isolate of SMR455
1629	RSH190	JC9387 Rif^{r} *leu*$^{+}$ *his*$^{+}$	SMR1618 x P1(SMR816); His^{+}
1630	RSH191	JC11450 Rif^{r} *leu*$^{+}$ *his*$^{+}$	SMR1619 x P1(SMR816); His^{+}
1631	RSH192	JC9388 Rif^{r} *leu*$^{+}$ *his*$^{+}$	SMR1620 x P1(SMR816); His^{+}
1633	MH129	*thyA rpoL thiA* [pMH101]	S. Sedgwick, MRC, London (Spanos and Sedgwick, 1984)
1634	MH130	*thyA rpoL thiA* [pHSG415]	"
1635	MH132	*polA1*(Ts) *thyA rpoL thiA* [pMH101]	"
1636	MH133	*polA1*(Ts) *thyA rpoL thiA* [pHSG415]	"
1637	MH135	background? [pMH101 *bla*::Tn*1000*]	"
1638	MH136	background? [pMH101 *polA*::Tn*1000*]	"
1639	MH147	*thr-1 leuB6 proA2 phr-1 recA1 argE3 thi-1 uvrA6 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44 gyrA98* [pMH101]	"
1640	MH148	*thr-1 leuB6 proA2 phr-1 recA1 argE3 thi-1 uvrA6 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44 gyrA98* [pHSG415]	"
1641	RK4349	*pro-3 Δlac-6 entA403 glnV44*(AS) *his-218 rpsL109 xylA5* or *xylA7 ilvC7 metE163*::Tn*10 metB1*	CGSC 6403; Tn at 86-87' linked to *polA*, *mutU*(*uvrD*)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1642	RSH193	JC9387 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$	SMR1629 x P1(SMR816); Lac$^+$
1643	RSH194	JC11450 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$	SMR1630 x P1(SMR816); Lac$^+$
1644	RSH195	JC9388 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$	SMR1631 x P1(SMR816); Lac$^+$
1648	RSH196	JC9387 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$	SMR1642 x P1(SMR816); Pro$^+$
1649	RSH197	JC11450 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$	SMR1643 x P1(SMR816); Pro$^+$
1650	RSH198	JC9388 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$	SMR1644 x P1(SMR816); Pro$^+$
1651	RSH199	C600 *thr*$^+$ *leu*$^+$ Rif^r *malB*::Tn*9* *ΔlacX74* *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ* *zah-281*::Tn*10*]	SMR1621 x P1(SMR785); Kan^r; LBH^s; **NOTE:** possibly heterozygous for *polA*
1652	RSH200	MG1655 Rif^r *malB*::Tn*9* *ΔlacX74* *ΔpolA*::*kan*(?) [F *proAB*$^+$ *lacI33ΩlacZ* *zah-281*::Tn*10*]	SMR1622 x P1(SMR785); Kan^r; LBH^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1653 (constructed independently)
1653	RSH201	MG1655 Rif^r *malB*::Tn*9* *ΔlacX74* *ΔpolA*::*kan*(?) [F *proAB*$^+$ *lacI33ΩlacZ* *zah-281*::Tn*10*]	SMR1622 x P1(SMR785); Kan^r; LBH^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1652 (constructed independently)
1658	RSH202	JC9387 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$	SMR1648 x P1(SMR816); Arg$^+$
1659	RSH203	JC11450 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$	SMR1649 x P1(SMR816); Arg$^+$
1660	RSH204	JC9388 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$	SMR1650 x P1(SMR816); Arg$^+$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1661	RSH205	M182 *ΔlacX74 malB*::Tn9 Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1465 x P1(SMR785); Kan^r; R17^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1472 & SMR1662 (constructed independently)
1662	RSH206	M182 *ΔlacX74 malB*::Tn9 Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1465 x P1(SMR785); Kan^r; R17^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1472 & SMR1661 (constructed independently)
1663	RSH207	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1472 x P1(SMR696); Mal$^+$; Cam^s; R17^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1664 (constructed independently), SMR1673 & SMR1674 (different P1 recipient)
1664	RSH208	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1472 x P1(SMR696); Mal$^+$; Cam^s; R17^s; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1663 (constructed independently), SMR1673 & SMR1674 (different P1 recipient)
1665	RSH209	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pMH101]	SMR1663 transformed with pMH101; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1667 (constructed independently)
1666	RSH210	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pHSG415]	SMR1663 transformed with pHSG415; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1668 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1667	RSH211	M182 *ΔlacX74* Rif^{r} *ΔpolA*::*kan*(?) [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*] [pMH101]	SMR1664 transformed with pMH101; Cam^{r}; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1665 (constructed independently)
1668	RSH212	M182 *ΔlacX74* Rif^{r} *ΔpolA*::*kan*(?) [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*] [pHSG415]	SMR1664 transformed with pHSG415; Cam^{r}; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1666 (constructed independently)
1670	RSH213	M182 *ΔlacX74* Rif^{r} [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1465 x P1(SMR696); Mal^{+}; Cam^{s}
1671	RSH214	MG1655 Rif^{r} *ΔlacX74* [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1622 x P1(SMR816); Mal^{+}; Cam^{s}
1672	RSH215	W3110 Rif^{r} *ΔlacX74* [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1623 x P1(SMR455); Mal^{+}; Cam^{s}
1673	RSH216	M182 *ΔlacX74* Rif^{r} *ΔpolA*::*kan*(?) [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1662 x P1(SMR696); Mal^{+}; Cam^{s}; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1663 & SMR1664 (constructed independently), SMR1674 (different P1 recipient)
1674	RSH217	M182 *ΔlacX74* Rif^{r} *ΔpolA*::*kan*(?) [F′ *proAB*$^{+}$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1661 x P1(SMR696); Mal^{+}; Cam^{s}; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1663 & SMR1664 (constructed independently), SMR1673 (different P1 recipient)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1675	RSH218	MG1655 Rif^r *ΔlacX74* *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*]	SMR1652 x P1(SMR816); Mal$^+$; Cam^s; **NOTE:** (i) possibly heterozygous for *polA*
1676	NK5525	*pro-81*::Tn*10 IN(rrnD-rrnE)1*	CGSC 6169; **NOTE:** this Tn is in *proA* or *proB*
1680	RSH220	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pMH101]	SMR1674 transformed with pMH101; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1665, SMR1667, SMR1682 (different parents)
1681	RSH221	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pHSG415]	SMR1674 transformed with pHSG415; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1666, SMR1668, SMR1683 (different parents)
1682	RSH222	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pMH101]	SMR1673 transformed with pMH101; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1665, SMR1667, SMR1680 (different parents)
1683	RSH223	M182 *ΔlacX74* Rif^r *ΔpolA*::*kan*(?) [F' *proAB*$^+$ *lacI33ΩlacZ zah-281*::Tn*10*] [pHSG415]	SMR1673 transformed with pHSG415; Cam^r; **NOTE:** (i) possibly heterozygous for *polA*; (ii) identical to SMR1666, SMR1668, SMR1681 (different parents)
1684	RSH224	JC9387 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$ *thr*$^+$	SMR1658 x P1(SMR816); Thr$^+$; prototroph
1685	RSH225	JC11450 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$ *thr*$^+$	SMR1659 x P1(SMR816); Thr$^+$; prototroph
1686	RSH226	JC9388 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$ *pro*$^+$ *arg*$^+$ *thr*$^+$	SMR1660 x P1(SMR816); Thr$^+$; prototroph

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1688	RSH227	W3110 *ΔpolA*::*kan* [pMH101]	SMR892 transformed with pMH101; Cam^r, LBH^r
1689	RSH228	W3110 *ΔpolA*::*kan* [pHSG415]	SMR892 transformed with pHSG415; Cam^r, LBH^s
1690	RSH229	C600 *ΔpolA*::*kan* [pMH101]	SMR893 transformed with pMH101; Cam^r, LBH^r
1691	RSH230	C600 *ΔpolA*::*kan* [pHSG415]	SMR893 transformed with pHSG415; Cam^r, LBH^s
1695	RSH231	M182 *ΔlacX74* Rif^r	Spontaneous Rif^r isolate of SMR1359
1698	RSH232	MG1655 Rif^r *proC* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1316 x P1(SMR696); Tet^r; Lac$^-$, Pro$^-$
1699	RSH233	W3110 Rif^r *proC* *zaj-3053*::Tn*10* *ΔlacX74*	SMR1327 x P1(SMR696); Tet^r; Lac$^-$, Pro$^-$
1702	RSH234	M182 *ΔlacX74* Rif^r *pro-81*::Tn*10*	SMR1695 x P1(SMR1676); Tet^r; Pro$^-$
1704	RSH235	FC29 *malB*::Tn*9*	SMR504 x P1(SMR533); Cam^r
1705	RSH236	FC40 *recA430* *recD6001*::*Tn10kan*	SMR508 x P1(SMR692); Kan^r; UV^r, RecD$^-$
1707	RSH237	MG1655 Rif^r *ΔlacX74*	SMR1698 x P1(SMR816); Pro$^+$; Lac$^-$, Tet^s
1708	RSH238	W3110 Rif^r *ΔlacX74*	SMR1699 x P1(SMR455); Pro$^+$; Lac$^-$, Tet^s
1709	RSH239	MG1655 Rif^r *ΔlacX74* *pro-81*::Tn*10*	SMR1707 x P1(SMR1676); Tet^r; Pro$^-$
1710	RSH240	W3110 Rif^r *ΔlacX74* *pro-81*::Tn*10*	SMR1708 x P1(SMR1676); Tet^r; Pro$^-$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1711	RSH241	M182 *ΔlacX74* Rif^r *pro-81*::Tn*10* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated SMR1702 x SMR506; Pro$^+$ & Tet^r; **NOTE:** not transducible with *ΔpolA*::*kan*
1713	SL1713	FC40 [pSL5]	Lab collection (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6)
1714	RSH242	MG1655 Rif^r *ΔlacX74* *pro-81*::Tn*10* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated SMR1709 x SMR506; Pro$^+$ & Tet^r; **NOTE:** not transducible with *ΔpolA*::*kan*
1715	RSH243	W3110 Rif^r *ΔlacX74* *pro-81*::Tn*10* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated SMR1710 x SMR506; Pro$^+$ & Tet^r; **NOTE:** difficult to transduce with *ΔpolA*::*kan* (see SMR1721)
1719	RSH244	FC40 *recA801 srl-300*::Tn*10*	SMR506 x P1(SMR85); Tet^r; Srl$^-$
1720	RSH245	FC40 *recG258*::Tn*10miniKan* *recA801 srl-300*::Tn*10*	SMR788 x P1(SMR85); Tet^r; Srl$^-$; **NOTE:** see SMR788
1721	RSH246	W3110 Rif^r *ΔlacX74* *pro-81*::Tn*10* *ΔpolA*::*kan*(?) [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1715 x P1(SMR785); Kan^r; LBH^r, UV^r; **NOTE:** should be LBH^s & UV^s; may be heterozygous for *polA*
1723	DE274	AB1157 *recA730* *srlC300*::Tn*10* *sulA211 pyrD*$^+$	R. Woodgate, NIH, Bethesda
1724	DE1242	AB1157 *Δ(gal-uvrB)301* *recA432 srlR301*::Tn*10* *sulA100*::Tn*5 pyrD*	"
1725	RW117	AB1157 *sulA100*::Tn*5 pyrD*	"

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1726	RSH247	FC40 *ruvA76*::Tn*10Sm*	SMR1609 cured of prophage by streaking on LBH and incubating at 43°C; Str^r, Tet^s, Kan^s, λ^s, λi21^s, UV^s (Harris *et al.*, 1996)
1728	RSH248	FC40 *ruvA76*::Tn*10Sm* *Δ(recA-srlR)306::Tn10*	SMR1726 x P1(SMR624); Tet^r; extremely UV^s
1729	RSH249	FC40 *recF*::Tn*3* *recD6001*::*Tn10kan* *Δ(recA-srlR)306::Tn10*	SMR1497 x P1(SMR624); Tet^r; extremely UV^s
1730	RSH250	FC40 *recD6001::Tn10kan* *recF*::Tn*3* *Δ(recA-srlR)306::Tn10*	SMR1496 x P1(SMR624); Tet^r; extremely UV^s
1743	RSH251	FC40 *ruvA200* *eda*::Tn*10*::λTSK	SMR1549 lysogenized with λTSK; Kan^r; Str^r, Tet^s; **NOTE:** grow at <32°C
1747	SL1747	FC40 [pSL6]	Lab collection (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6)
1749	RSH252	FC40 *ruvC53 eda*::Tn*10*::λTSK	SMR789 lysogenized with λTSK; Kan^r; Str^r, Tet^s; **NOTE:** grow at <32°C
1750	RSH253	JC9387 Rif^r *leu*$^+$ *his*$^+$	SMR1618 x P1(SMR123); His$^+$
1752	RSH254	MG1655 Rif^r *ΔlacX74* *zah-281*::Tn*10*	SMR1707 x P1(SMR862); Tet^r; Lac$^-$
1754	RSH255	FC40 *ruvA76*::Tn*10Sm* *Δ(recA-srlR)306::Tn10* (λ*precA*)	SMR1728 lysogenized with λ*precA* ; Mal$^+$ & UV^r at 30°C; λ^r, λi21^s; **NOTE:** grow at <32°C
1755	RSH256	W3110 Rif^r *ΔlacX74* *pro-81*::Tn*10* [F'*proAB*$^+$ *lacI33ΩlacZ*] [pHSG415]	SMR1715 transformed with pHSG415; Cam^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1756	RSH257	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10* [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101]	SMR1715 transformed with pMH101; Cam^{r}
1757	RSH258	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10* [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101 *bla*::Tn*1000*]	SMR1715 transformed with [pMH101 *bla*::Tn*1000*]; Cam^{r}
1758	RSH259	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10* [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101 *polA*::Tn*1000*]	SMR1715 transformed with [pMH101 *polA*::Tn*1000*]; Cam^{r}
1759	RSH260	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10 ΔpolA*::*kan*(?) [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pHSG415]	SMR1721 transformed with pHSG415; Cam^{r}; **NOTE:** see SMR1721
1760	RSH261	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10 ΔpolA*::*kan*(?) [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101]	SMR1721 transformed with pMH101; Cam^{r}; **NOTE:** see SMR1721
1761	RSH262	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10 ΔpolA*::*kan*(?) [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101 *bla*::Tn*1000*]	SMR1721 transformed with [pMH101 *bla*::Tn*1000*]; Cam^{r}; **NOTE:** see SMR1721
1762	RSH263	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10 ΔpolA*::*kan*(?) [F'*proAB*$^{+}$ *lacI33ΩlacZ*] [pMH101 *polA*::Tn*1000*]	SMR1721 transformed with [pMH101 *polA*::Tn*1000*]; Cam^{r}; **NOTE:** see SMR1721
1763	RSH264	W3110 Rif^{r} *ΔlacX74 pro-81*::Tn*10 recB21 recD6001::Tn10kan* [F'*proAB*$^{+}$ *lacI33ΩlacZ*]	SMR1715 x P1(SMR738); Kan^{r}; moderately UV^{s}, RecD^{-}; **NOTE:** identical to SMR1764 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1764	RSH265	W3110 Rif^r *ΔlacX74 pro-81*::Tn*10* *recD6001*::*Tn10kan* [F′*proAB*$^+$ *lacI33ΩlacZ*]	SMR1715 x P1(SMR738); Kan^r; UV^r, RecD$^-$; **NOTE:** identical to SMR1763 (constructed independently)
1766	RSH266	JC9387 Rif^r *leu*$^+$ *his*$^+$ *lac*$^+$	SMR1750 x P1(SMR816); Lac$^+$
1767	RSH267	FC40 *ruvC53 eda*::Tn*10*(Tet^s)	SMR1749 cured of prophage by streaking on LBH and incubating at 43°C; λ^s, λi21^s, moderately UV^s; Tet^s, Kan^s, Str^s; **NOTE:** probably an imprecise excision of λ
1770	RSH268	JC9387 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$	SMR1766 x P1(SMR816); Arg$^+$; Rif^S
1771	RSH269	JC11450 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$	SMR1643 x P1(SMR816); Arg$^+$; Rif^S
1772	RSH270	JC9388 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$	SMR1644 x P1(SMR816); Arg$^+$; Rif^S
1777	RSH271	FC40 *ruvA76*::Tn*10Sm* *Δ(recA-srlR)306*::*Tn10* *recG258*::Tn*10miniKan* (λ*precA*)	SMR1754 x P1(SMR788) (30°C); Kan^r; extremely UV^s; **NOTE:** grow at <32°C
1778	RSH272	JC9387 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$ *thr*$^+$	SMR1770 x P1(SMR816); Thr$^+$
1779	RSH273	JC11450 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$ *thr*$^+$	SMR1771 x P1(SMR816); Thr$^+$
1780	RSH274	JC9388 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$ *thr*$^+$	SMR1772 x P1(SMR816); Thr$^+$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1781	RSH275	FC40 *ruvA76*::Tn*10Sm* *Δ(recA-srlR)306*::*Tn10* *recG258*::Tn*10miniKan*	SMR1777 cured of prophage by streaking on LBH and incubating at 43°C; λ^s, λi21^s, extremely UV^s [(Harris *et al.*, 1996) & CHAPTER 3]
1782	RSH276	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74 zah-281*::Tn*10* Rif^r	SMR1778 x P1(SMR1752); Tet^r; Lac$^-$; **NOTE:** spontaneously Rif^r
1783	RSH277	FC40 *ruvC53 eda*::Tn*10*(Tet^s) *Δ(recA-srlR)306*::*Tn10*	SMR1767 x P1(SMR624); Tet^r; extremely UV^s
1784	RSH278	FC40 *ruvC53 eda*::Tn*10*(Tet^s) *recG258*::Tn*10miniKan*	SMR1767 x P1(SMR788); Kan^r; extremely UV^s
1785	RSH279	JC11450 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$ *thr*$^+$ Rif^r	Spontaneous Rif^r isolate of SMR1779
1788	RSH280	JC9388 *leu*$^+$ *his*$^+$ *lac*$^+$ *arg*$^+$ *thr*$^+$ Rif^r	Spontaneous Rif^r isolate of SMR1780
1789	RSH281	FC40 *ruvC53 eda*::Tn*10*(Tet^s)	SMR789 subjected to Tet^s selection (Maloy and Nunn, 1981); Tet^s; **NOTE:** unable to transduce this strain - may be resistant to P1 infection
1790	SL1790	FC40 [pSL4]	Lab collection (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6)
1791	SL1791	FC40 [pSL7]	Lab collection (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6)
1797	RSH282	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74 zah-281*::Tn*10* Rif^r	SMR1785 x P1(SMR1752); Tet^r; Lac$^-$
1798	RSH283	FC40 *ruvC53 eda*::Tn*10*(Tet^s) *Δ(recA-srlR)306*::*Tn10* (λ*precA*)	SMR1783 lysogenized with λ*precA* ; Mal$^+$ & UV^r at 30°C; λ^r, λi21^s; **NOTE:** grow at <32°C

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1799 to 1803	RSH284 to RSH288	FC40 *ruvA59*::Tn*10* Lac$^+$	Spontaneous Lac$^+$ isolates 1-4, 6 of SMR1551; moderately UV^s
1804 to 1808	RSH289 to RSH293	FC40 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan* Lac$^+$	Spontaneous Lac$^+$ isolates 1, 2, 4-6 of SMR1564; extremely UV^s
1809	RSH294	FC40 *sulA100*::Tn*5 pyrD*	FC40 x P1(SMR1725); Kan^r; Pyr$^-$
1810	RSH295	FC40 *sulA100*::Tn*5*	FC40 x P1(SMR1725); Kan^r; Pyr$^+$
1811 to 1814	RSH296 to RSH299	FC40 *ruvC53 eda-51::Tn10* Lac$^+$	Spontaneous Lac$^+$ isolates 1-4 of SMR789; moderately UV^s
1815 to 1818	RSH300 to RSH303	FC40 *ruvC53 eda-51::Tn10* *recG258*::Tn*10miniKan* Lac$^+$	Spontaneous Lac$^+$ isolates 1-4 of SMR799; extremely UV^s
1819 to 1822	RSH304 to RSH307	FC40 *recG258*::Tn*10miniKan* Lac$^+$	Lac$^+$ isolates (possibly adaptive), 43-G21, G44, G5, G7 of SMR788; **NOTE:** see SMR788
1827	RSH308	FC40 *sulA211*	SMR1809 x P1(SMR1723); Pyr$^+$; Kan^s
1828	RSH309	FC40 *ruvC53 eda*::Tn*10*(Tet^s) *Δ(recA-srlR)306::Tn10* *recG258*::Tn*10miniKan* (λ*precA*)	SMR1798 x P1(SMR788) (30°C); Kan^r; extremely UV^s; **NOTE:** grow at <32°C
1831	RSH310	FC40 *ruvC53 eda*::Tn*10*(Tet^s) *Δ(recA-srlR)306::Tn10* *recG258*::Tn*10miniKan*	SMR1828 cured of prophage by streaking on LBH and incubating at 43°C; λ^s, λi21^s, extremely UV^s
1832	RSH311	FC40 *sulA211 recA730*(?) *srlC300*::Tn*10*	SMR1827 x P1(SMR1723); Tet^r; **NOTE:** *recA730* not verified

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1833	RSH312	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* [F'*proAB*$^+$ *lacI33*Ω*lacZ*]	Mated SMR1797 x SMR506; Pro$^+$ & Tet^r
1953	RSH313	FC40 *recJ*::Tn*10*::λTSK	SMR690 lysogenized with λTSK; Kan^r; Str^r, Tet^s; **NOTE:** grow at <32°C
1954	RSH314	FC40 *sulA211 recF*::Tn*3*	SMR1827 x P1(SMR686); Amp^r; moderately UV^s, but not quite as sensitive as *recF* (SMR686)
1955	RSH315	FC40 *sulA211 recA730*(?) *srlC300*::Tn*10* *recF*::Tn*3*	SMR1832 x P1(SMR686); Amp^r; slightly UV^s, but more resistant than *recF* (SMR686); **NOTE:** *recA730* not verified
1982	RSH316	FC40 *recG258*::Tn*10miniKan*	SMR506 x P1(SMR600); Kan^r; slightly UV^s; **NOTE:** this strain is a *bona fide* *recG* mutant (Harris *et al.*, 1996; CHAPTER 3)
1983	RSH317	FC40 *ruvA59*::Tn*10*	SMR506 x P1(SMR603); Tet^r; moderately UV^s; **NOTE:** identical to SMR1551 (constructed independently)
1984	RSH318	FC40 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan*	SMR1983 x P1(SMR1982); Kan^r; extremely UV^s; **NOTE:** identical to SMR1551 (different P1 recipients) identical to SMR1564 (different P1 donor), SMR2036 & SMR2037 (constructed independently)
1985	RSH319	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recF*::Tn*3* [F'*proAB*$^+$ *lacI33*Ω*lacZ*]	SMR1833 x P1(SMR686); Amp^r; moderately UV^s

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
1986	RSH320	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recQ61*::Tn*3* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR589); Amp^r; moderately UV^s
1987	RSH321	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recN1502*::Tn*5* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR732); Kan^r; UV^r
1988	RSH322	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recO1504*::Tn*5* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR733); Kan^r; moderately UV^s
1989	RSH323	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recR252*::Tn*10-9* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR731); Kan^r; moderately UV^s
1990	RSH324	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recD6001::Tn10kan* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR738); Kan^r; UV^r, RecD$^-$
1991	RSH325	JC11450 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ Rif^r *ΔlacX74 zah-281*::Tn*10* *recB21 recD6001::Tn10kan* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR1833 x P1(SMR738); Kan^r; moderately UV^s, RecD$^-$
2001	RSH326	FC40 *recG258*::Tn*10miniKan* *Δ(recA-srlR)306::Tn10*	SMR1982 x P1(SMR624); Tet^r; extremely UV^s (Harris *et al.*, 1996; CHAPTER 3)
2002	NR9360	*ara-9 fhuA1 lacY1*(or *lacZ4*?) *tsx-3 supE44 galK2 hisG4*(Oc) *rfbD1*(?) *trp-3*(Oc) *rpsL8*(or *rpsL9*(?) *malA1 mtl-1 metE46 thi-1 mutL211*::Tn*5*	R.M. Schaaper, NIEHS, Research Triangle Park, North Carolina
2003	NR9915	NR9360 *zae-502*::Tn*10* *dnaE915 zae*::Tn*10d*-Cam	" NR9360 is SMR2002

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2004	NR9918	NR9360 *zae-502*::Tn*10* *dnaE*$^+$ *zae*::Tn*10d*-Cam	"
2005	RSH327	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated SMR1782 x SMR506; Pro$^+$ & Tet^r
2006	RSH328	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r *recF*::Tn*3* [F*proAB*$^+$ *lacI33ΩlacZ*]	SMR2005 x P1(SMR686); Amp^r; extremely UV^s
2007	RSH329	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r *recQ61*::Tn*3* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR2005 x P1(SMR589); Amp^r; UV^r; **NOTE:** this strain should be UV^s, therefore it may have a suppressor
2008	RSH330	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r *recN1502*::Tn*5* [F'*proAB*$^+$ *lacI33ΩlacZ*]	SMR2005 x P1(SMR732); Kan^r; UV^r; **NOTE:** this strain should be UV^s, therefore it may have a suppressor
2009	RSH331	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r *recO1504*::Tn*5* [F*proAB*$^+$ *lacI33ΩlacZ*]	SMR2005 x P1(SMR733); Kan^r; extremely UV^s
2010	RSH332	JC9387 *leu*$^+$ *his*$^+$ *arg*$^+$ *thr*$^+$ *ΔlacX74* *zah-281*::Tn*10* Rif^r *recR252*::Tn*10-9* [F*proAB*$^+$ *lacI33ΩlacZ*]	SMR2005 x P1(SMR731); Kan^r; extremely UV^s
2011	RSH333	JC11450 *recF*::Tn*3*	SMR122 x P1(SMR686); Amp^r; moderately UV^s
2025	RSH334	FC40 *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10*	SMR506 x P1(SMR2003); Cam^r; Tet^r (Harris *et al.*, 1997a; CHAPTER 4)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2026	RSH335	FC40 *zae*::Tn*10d*-Cam *dnaE*$^{+}$ *zae-502*::Tn*10*	SMR506 x P1(SMR2004); Cam^{r}; Tet^{r} (Harris *et al.*, 1997a; CHAPTER 4)
2027	RSH336	JC9387 [F'*proAB*$^{+}$ *lacI33ΩlacZ*]	Mated SMR121 x SMR506; Pro^{+} & Str^{r}; R17^{s}
2028 to 2033	RSH337 to RSH342	FC40 *recG258*::Tn*10miniKan* Lac^{+}	Spontaneous Lac^{+} isolates 1-6 of SMR1982; slightly UV^{s}
2034	RSH343	FC40 *ruvA200 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR1549 x P1(SMR1982); Kan^{r}; extremely UV^{s}; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1563 (different P1 donor) and SMR2035 (constructed independently)
2035	RSH344	FC40 *ruvA200 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR1549 x P1(SMR1982); Kan^{r}; extremely UV^{s}; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1563 (different P1 donor) and SMR2034 (constructed independently)
2036	RSH345	FC40 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan*	SMR1551 x P1(SMR1982); Kan^{r}; extremely UV^{s}; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1564 (different P1 donor), SMR1984 (different P1 recipient) and SMR2037 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2037	RSH346	FC40 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan*	SMR1551 x P1(SMR1982); Kan^r; extremely UV^s; constructed at <32°C to **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1564 (different P1 donor), SMR1984 (different P1 recipient) and SMR2036 (constructed independently)
2038	RSH347	FC40 *ruvB9 zea-3*::Tn*10* *recG258*::Tn*10miniKan*	SMR1552 x P1(SMR1982); Kan^r; extremely UV^s; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1565 (different P1 donor) and SMR2039 (constructed independently)
2039	RSH348	FC40 *ruvB9 zea-3*::Tn*10* *recG258*::Tn*10miniKan*	SMR1552 x P1(SMR1982); Kan^r; extremely UV^s; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR1565 (different P1 donor) and SMR2038 (constructed independently)
2040	RSH349	FC40 *ruvC53 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR789 x P1(SMR1982); Kan^r; extremely UV^s; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR799 (different P1 donor) and SMR2041 (constructed independently)
2041	RSH350	FC40 *ruvC53 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR789 x P1(SMR1982); Kan^r; extremely UV^s; **NOTE:** (i) constructed at <32°C to avoid picking up suppressors; (ii) identical to SMR799 (different P1 donor) and SMR2040 (constructed independently)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2042	RSH351	FC29 *ruvA59*::Tn*10*	SMR504 x SMR603; Tet^r; moderately UV^s
2043	RSH352	FC29 *eda-51*::*Tn10*	SMR504 x SMR601; Tet^r; UV^r
2044	RSH353	FC29 *ruvC53 eda-51*::*Tn10*	SMR504 x SMR601; Tet^r; moderately UV^s (Harris *et al.*, 1996; CHAPTER 3)
2052	RSH354	FC29 *ruvA59*::Tn*10* *recG258*::Tn*10miniKan*	SMR2042 x P1(SMR1982); Kan^r; extremely UV^s
2053	RSH355	FC29 *ruvC53 eda-51*::*Tn10* *recG258*::Tn*10miniKan*	SMR2044 x P1(SMR1982); Kan^r; extremely UV^s (Harris *et al.*, 1996; CHAPTER 3)
2054	RSH356	FC40 *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10* *mutL211*::Tn*5*	SMR2025 x P1(SMR620); Kan^r; strong mutator (Lac$^+$, Nal^r) (Harris *et al.*, 1997a; CHAPTER 4)
2055	RSH357	FC40 *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10* [*mutS201*::Tn*5*(?)]	SMR2025 x P1(SMR622); Kan^r; **NOTE:** weak- or non-mutator (Lac$^+$, Nal^r), therefore SMR622 may not be *mutS*::Tn*5*; see SMR2075 for a *bona fide dnaE915 mutS* strain
2056	RSH358	FC40 *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae-502*::Tn*10* *mutL211*::Tn*5*	SMR2026 x P1(SMR620); Kan^r; strong mutator (Lac$^+$, Nal^r) (Harris *et al.*, 1997a; CHAPTER 4)
2057	RSH359	FC40 *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae-502*::Tn*10* [*mutS201*::Tn*5*(?)]	SMR2026 x P1(SMR622); Kan^r; **NOTE:** weak- or non-mutator (Lac$^+$, Nal^r), therefore SMR622 may not be *mutS*::Tn*5*; see SMR2075 for a *bona fide dnaE915 mutS* strain

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2073	CSM61	*polA1 polB100 polC1026 leu his thy pcbA1 supE44*(=SuII$^+$)	R. Moses, Oregon Health Sciences University (Bryan and Moses, 1984)
2074	61P-14	*dnaE511 pcbA1 leu his thy zic-1*::Tn*10*	"
2075	RSH360	FC40 *zae*::Tn*10d*-Cam *dnaE915 zae-502*::Tn*10* *mutS201*::Tn*5*	SMR2025 x P1(SMR438); Kan^r; strong mutator (Lac$^+$) (Harris *et al.*, 1997a; CHAPTER 4)
2076	RSH361	FC40 *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae-502*::Tn*10* *mutS201*::Tn*5*	SMR2026 x P1(SMR438); Kan^r; strong mutator (Lac$^+$) (Harris *et al.*, 1997a; CHAPTER 4)
2077	831	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 mal Δ(lac-proAB)* [F' *proAB*$^+$ *lacI*^{q} *traD36 ΔlacZ*(M15)] [pHN3]	C. McHenry; University of Colorado, Denver
2078	471	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 mal Δ(lac-proAB)* [F' *proAB*$^+$ *lacI*^{q} *traD36 ΔlacZ*(M15)] [pDNAE OPIΔ*Pst*I-*Apa*LI]	"
2079	RSH362	FC40 [pHC5]	SMR506 transformed with pHC5; Amp^r
2080	RSH363	FC40 [pHC5Δ*Hinc*II]	SMR506 transformed with pHC5Δ*Hinc*II; Amp^r
2081	RSH364	AB1157 *Δ(srlR-recA)304* [pHC5]	SMR471 transformed with pHC5; Amp^r
2082	RSH365	AB1157 *Δ(srlR-recA)304* [pHC5Δ*Hinc*II]	SMR471 transformed with pHC5Δ*Hinc*II; Amp^r
2208	RSH366	FC40 [pHN3]	SMR506 transformed with pHN3; Amp^r
2209	RSH367	FC40 [pDNAE OPIΔ*Pst*I-*Apa*LI]	SMR506 transformed with [pDNAE OPIΔ*Pst*I-*Apa*LI]; Amp^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2333 to 2337	RSH368 to RSH372	FC40 *recG258*::Tn*10miniKan* *Δ(recA-srlR)306*::*Tn10* Lac$^+$	Spontaneous Lac$^+$ isolates a-e of SMR2001; **NOTE:** "d", SMR2336, is Tet^s (Harris *et al.*, 1996; CHAPTER 3)
2338	RSH373	FC29 *recB21* *recD6001*::*Tn10kan*	SMR504 x P1(SMR738); Kan^r; moderately UV^s
2339	RSH374	FC29 *recD6001*::*Tn10kan*	SMR504 x P1(SMR738); Kan^r; UV^r
2340	RSH375	FC29 *recG258*::Tn*10miniKan*	SMR504 x P1(SMR600); Kan^r; slightly UV^s
2448 to 2451	RSH376 to RSH379	FC40 *recB21* Lac$^+$	Spontaneous Lac$^+$ isolates a-d of SMR593; moderately UV^s
2560	RSH380	FC29 *mutL211*::Tn*5*	SMR504 x P1(SMR620); Kan^r; mutator (Rif^r)
2561	RSH381	FC29 *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10*	SMR504 x P1(SMR2003); Cam^r; Tet^r
2562	RSH382	FC29*zae*::Tn*10d*-Cam *dnaE*$^+$ *zae-502*::Tn*10*	SMR504 x P1(SMR2004); Cam^r; Tet^r
2580	RSH383	FC29 *mutS215*::Tn*10*	SMR504 x P1(SMR685); Tet^r; mutator (Rif^r and Nal^r)
2581	RSH384	FC40 *ruvA76*::Tn*10Sm* *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10*	SMR1726 x P1(SMR2003); Cam^r; Tet^r
2582	RSH385	FC40 *ruvA76*::Tn*10Sm* *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae-502*::Tn*10*	SMR1726 x P1(SMR2004); Cam^r; Tet^r
2583	RSH386	FC40 *ruvA76*::Tn*10Sm* *zae*::Tn*10d*-Cam *dnaE915* *zae-502*::Tn*10* *recG258*::Tn*10miniKan*	SMR2581 x P1(SMR1982); Kan^r; extremely UV^s

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2584	RSH387	FC40 *ruvA76*::Tn*10Sm* *zae*::Tn*10d*-Cam *dnaE*⁺ *zae-502*::Tn*10* *recG258*::Tn*10miniKan*	SMR2582 x P1(SMR1982); Kan^r; extremely UV^s
2585	RSH388	FC40 *sulA100*::Tn*5* *recF*::Tn*3*	SMR1810 x P1(SMR686); Amp^r; moderately UV^s, like *recF* (SMR686)
2586	RSH389	FC40 *sulA100*::Tn*5* *recA730* *srlC300*::Tn*10*	SMR1810 x P1(SMR1723); Tet^r; UV^r, like SMR506
2587	RSH390	FC40 *sulA100*::Tn*5* *recF*::Tn*3* *recA730* *srlC300*::Tn*10*	SMR2585 x P1(SMR1723); Tet^r; more UV^r than *recF*(SMR2585) but less than SMR1723 - i.e. *recA730* appears to be a partial suppressor of *recF*; **NOTE:** identical to SMR2588 (different parents)
2588	RSH391	FC40 *sulA100*::Tn*5* *recA730* *srlC300*::Tn*10* *recF*::Tn*3*	SMR2586 x P1(SMR686); Amp^r; more UV^r than *recF*(SMR686) but less than SMR2586 - i.e. *recA730* appears to be a partial suppressor of *recF*; **NOTE:** identical to SMR2587 (different parents)
2597	SZ2597	FC40 *Δ(xseA-guaB)* *zff-3139*::Tn*10kan*	Lab collection (Harris *et al.*, 1997c)
2598	AM561	*ΔruvAC65 eda-51*::*Tn10*	R.G. Lloyd, University of Nottingham, U.K.
2599	RSH392	FC40 *lexA3 malB*::Tn*9* *recD6001*::*Tn10kan*	SMR841 x P1(SMR692); Kan^r; UV^s like *lexA3* (SMR841); **NOTE:** identical to SMR3087 (except selection for Kan^r was not maintained during construction & different P1 donor)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
2600	RSH393	FC40 *lexA3 recD6001*::*Tn10kan*	SMR868 x P1(SMR692); Kan^r; UV^S like *lexA3* (SMR868); **NOTE:** identical to SMR3088 (except selection for Kan^r was not maintained during construction & different P1 donor)
2601	RSH394	FC40 *sulA211 lexA71*::Tn*5*	SMR1827 x P1(SMR878); Kan^r
2602	RSH395	FC40 *sulA211 recF*::Tn*3* *lexA71*::Tn*5*	SMR1954 x P1(SMR878); Kan^r; appears slightly more UV^r than SMR1954
3004	pBLW20	DH5α [pBLW20]	M. Cox, University of Wisconsin, Madison (Webb *et al.*, 1995)
3005	pET21d	DH5α [pET21d]	"
3006	AB1976	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1*	CGSC 1976
3007	RS3087	Hfr(*valS*--<--*attP4*) *relA1 spoT1 fad-71*::Tn*10 thi-1*	CGSC 6344
3009	SZ3009	FC40 *Δ(recA-srlR)306*::*Tn10* [pSL4]	Lab collection (Harris **et al.**, 1997b; CHAPTER 6)
3010	SZ3010	FC40 *Δ(recA-srlR)306*::*Tn10* [pSL7]	Lab collection (Harris **et al.**, 1997b; CHAPTER 6)
3011	SZ3011	FC40 *Δ(xseA-guaB) zff-3139*::Tn*10kan* *ΔxonA300*::*CAT*	Lab collection
3013 to 3018	RSH396 to RSH401	FC40 *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates a-f of SMR3009 (Harris *et al.*, 1997b; CHAPTER 6)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3019 to 3024	RSH402 to RSH407	FC40 *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates a-f of SMR3010 (Harris *et al.*, 1997b; CHAPTER 6)
3027	CAG 18496	MG1655 *fadAB101*::Tn*10*	C. Gross, UCSF; Tn at 86.25' linked to *polA* (Singer *et al.*, 1989)
3028	CAG 18557	MG1655 *fadAB3165*::Tn*10kan*	C. Gross, UCSF; Tn at 86.25' linked to *polA*; (Singer *et al.*, 1989)
3029	CAG 18495	MG1655 *zih-35*::Tn*10*	C. Gross, UCSF; Tn at 87' linked to *polA* (Singer *et al.*, 1989)
3030	CAG 18601	MG1655 *zih-3088*::Tn*10Kan*	C. Gross, UCSF; Tn at 87' linked to *polA* (Singer *et al.*, 1989)
3031	CAG 18636	MG1655 *zii-3088*::Tn*10Kan*	C. Gross, UCSF; Tn at 87.5' linked to *polA* (Singer *et al.*, 1989)
3032 to 3037	RSH408 to RSH413	FC40 Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 4-1, 6, 7, 13, 21, 24 of SMR1790 (Harris *et al.*, 1997b; CHAPTER 6)
3038 to 3043	RSH414 to RSH419	FC40 Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 7-4, 19, 20, 21, 24, 37 of SMR1791 (Harris *et al.*, 1997b; CHAPTER 6)
3044	RSH420	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 fad-71*::Tn*10*	SMR3006 x P1(SMR3007); Tet^r; Tn is linked to *polA*$^+$
3045	RSH421	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 mutL211*::Tn5	SMR3006 x P1(SMR620); Kan^r; mutator (Gal$^+$)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3046	AQ9247	*his-29 trpA9605 pro ilv metB deoB*(or *C*) *thyA priA1*::*kan spa-47*	T. Kogoma, New Mexico; *priA1*::*kan* is a deletion-insertion mutation (Kogoma *et al*., 1996; Lee and Kornberg, 1991)
3047	RSH422	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 fad-71*::Tn*10 mutL211*::Tn*5*	SMR3044 x P1(SMR620); Kan^r; mutator (Gal$^+$)
3048	RSH423	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 fad-71*::Tn*10 zac-3093*::Tn*10kan*	SMR3044 x P1(SMR860); Kan^r; Tn at 2' linked to *polB*
3049	RSH424	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 fad-71*::Tn*10 zid-3162*::Tn*10kan*	SMR3044 x P1(SMR864); Kan^r; Tn at 83' linked to *recF*
3050 to 3052	RSH425 to RSH427	FC40 *priA2*::*kan* Lac$^+$	Spontaneous Lac$^+$ isolates #1 -3 of SMR1489
3060	RSH428	FC40 [pBLW20]	SMR506 transformed with pBLW20; Amp^r
3061	RSH429	FC40 [pET21d]	SMR506 transformed with pET21d; Amp^r
3062	RSH430	FC40 *lexA3 malB*::Tn*9* [pBLW20]	SMR841 transformed with pBLW20; Amp^r
3063	RSH431	FC40 *lexA3 malB*::Tn*9* [pET21d]	SMR841 transformed with pET21d; Amp^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3064	RSH432	FC40 *lexA3* [pBLW20]	SMR868 transformed with pBLW20; Amp^r
3065	RSH433	FC40 *lexA3* [pET21d]	SMR868 transformed with pET21d; Amp^r
3066	RSH434	FC40 [pMH101]	SMR506 transformed with pMH101; Cam^r; **NOTE:** identical to SMR3068 (constructed independently)
3067	RSH435	FC40 [pHSG415]	SMR506 transformed with pHSG415; Cam^r; **NOTE:** identical to SMR3069 (constructed independently)
3068	RSH436	FC40 [pMH101]	SMR506 transformed with pMH101; Cam^r; **NOTE:** identical to SMR3066 (constructed independently)
3069	RSH437	FC40 [pHSG415]	SMR506 transformed with pHSG415; Cam^r; **NOTE:** identical to SMR3067 (constructed independently)
3070	RSH438	FC40 *xonA300::CAT*	FC40 x P1(SMR839); Cam^r; His^+
3071	RSH439	FC40 *recD6001::Tn10kan*	FC40 x P1(SMR577); Kan^r; UV^r, $RecD^-$; **NOTE:** identical to SMR692 (except selection for Kan^r was maintained during construction)
3072	SZ3072	FC40 *recD1014*	Lab collection
3073	RSH440	FC36 *fad-71*::Tn*10*	SMR505 x P1(SMR3007); Tet^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3074	RSH441	*ara-9 fhuA21 Δ(gpt-proA)62 lacY1*(or *lacZ4*) *tsx-3 glnV44*(AS) *galK2 trpE3*(Oc) *hisG4*(Oc) *rfbD1*(?) *rpsL8*(or *rpsL9*) *malT1 mtl-1 metE46 thi-1 fadAB3165*::Tn*10kan*	SMR3006 x P1(SMR3028); Kan^r
3075	RSH442	FC40 [pMH101] [pSL4]	SMR3066 transformed with pSL4; Kan^r; Cam^r
3076 to 3085	RSH443 to RSH452	FC40 *priA2*::*kan* Lac$^+$	Spontaneous Lac$^+$ isolates 4-13 of SMR1489
3086	RSH453	FC40 *mutL211*::Tn*5* [pW17]	SMR620 transformed with pW17; Amp^r; Kan^r; **NOTE:** see SMR620
3087	RSH454	FC40 *lexA3 malB*::Tn*9 recD6001*::*Tn10kan*	SMR841 x P1(SMR3071); Kan^r; UV^s like *lexA3* (SMR841); **NOTE:** identical to SMR2599 (except selection for Kan^r was maintained during construction & different P1 donor)
3088	RSH455	FC40 *lexA3 recD6001*::*Tn10kan*	SMR868 x P1(SMR3071); Kan^r; UV^s like *lexA3* (SMR868); **NOTE:** identical to SMR2600 (except selection for Kan^r was maintained during construction & different P1 donor)
3089	SZ3089	FC40 *ΔxonA300*::*CAT*	Lab collection
3090	SZ3090	FC40 *ΔxonA300*::*CAT recJ284*::Tn*10*	Lab collection; **NOTE:** identical to SMR3469 & SMR3470 (different P1 donors)
3091	SMR3091	JC3272 [pOXTc]	L. Frost, University of Alberta

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3092	RSH456	FC40 [pMH101 *polA*::Tn*1000*]	SMR506 transformed with [pMH101 *polA*::Tn*1000*]; Cam^r
3099	RSH457	FC40 [pMH101 *bla*::Tn*1000*]	SMR506 transformed with [pMH101 *bla*::Tn*1000*]; Cam^r
3100	RSH458	FC36 *priA1*::*kan*	SMR505 x P1(SMR3046); Kan^r; LBH^s
3105	RSH459	FC40 [pMH101 *polA*::Tn*1000*] [pSL4]	SMR3092 transformed with [pSL4]; Kan^r; Cam^r
3106	RSH460	FC40 [pMH101 *bla*::Tn*1000*] [pSL4]	SMR3099 transformed with [pSL4]; Kan^r; Cam^r
3109	SZ3109	FC40 *recD1014* [pSL4]	Lab collection (Harris *et al.*, 1997b; CHAPTER 6)
3110	SZ3110	FC40 *recD1014* [pSL7]	"
3111	SZ3111	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10* [pSL4]	"
3112	SZ3112	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10*[pSL7]	"
3114	RSH461	FC36 *zac-3093*::Tn*10kan*	SMR505 x P1(SMR860); Kan^r; Pro$^-$, Lac$^-$
3115	RSH462	FC36 *zid-3162*::Tn*10kan*	SMR505 x P1(SMR864); Kan^r; Pro$^-$, Lac$^-$
3116	KIM1	FC40 *ΔxonA300*::*CAT* *Δ(recA-srlR)306*::*Tn10*	SMR3070 x P1(SMR624); Tet^r; Cam^r, extremely UV^s
3117	KIM2	FC40 *ΔxonA300*::*CAT* *recB21 argA*::Tn*10*	SMR3070 x P1(SMR580); Tet^r; Cam^r, moderately UV^s
3118	KIM3	FC40 *ΔxonA300*::*CAT* *argA*::Tn*10*	SMR3070 x P1(SMR580); Tet^r; Cam^r, UV^r
3119	KIM4	FC40 *ΔxonA300*::*CAT* *ruvC53* *eda-51*::*Tn10*	SMR3070 x P1(SMR601); Tet^r; Cam^r, moderately UV^s

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3120	KIM5	FC40 *xonA300*::*CAT* *eda-51*::*Tn10*	SMR3070 x P1(SMR601); Tet^r; Cam^r, UV^r
3121	KIM6	FC40 *xonA300*::*CAT* *ruvA59*::Tn*10*	SMR3070 x P1(SMR603); Tet^r; Cam^r, moderately UV^s
3122	RM5268	MG1655 *eda-57*::Tn*10*::*Cam*	R. Maurer, Case Western Reserve University (Foster *et al.*, 1996)
3123	RM4714	MG1655 *recG162* *zib-636*::Tn*10*	"
3130	JC19018	*Δ(lac-proAB)*$_{\mathrm{XIII}}$ *hisG4 argE3 thr-1 ara-14 xyl-5 mtl-1 rpsL31 sulA*::Mu *d*(Ap, *lac*, *B*::Tn*9*) *priA2*::*kan dnaC809 zjj-202*::Tn*10*	S. Sandler, Berkeley
3131	CAG 18429	MG1655 *zji-6*::Tn*10*	C. Gross, UCSF; Tn at 98.25' linked to *dnaC* (Singer *et al.*, 1989)
3132	CAG 18430	MG1655 *zjj-202*::Tn*10*	C. Gross, UCSF; Tn at 99.5' linked to *dnaC* (Singer *et al.*, 1989)
3133	RSH463	FC29 *priA2*::*kan*	SMR504 x P1(SMR840); Kan^r; UV^s, LBH^s
3134	RSH464	FC29 *priA1*::*kan*	SMR504 x P1(SMR3046); Kan^r; UV^s, LBH^s
3135	RSH465	FC36 *priA2*::*kan*	SMR505 x P1(SMR840); Kan^r; LBH^s
3136	RSH466	FC40 *sulA211 recD1903*::Tn*10*	SMR1827 x P1(SMR582); Tet^r; RecD$^-$
3137	RSH467	FC40 *sulA211 recF*::Tn*3* *recD1903*::Tn*10*	SMR1954 x P1(SMR1954); Tet^r; RecD$^-$
3138	RSH468	FC40 *sulA211 lexA71*::Tn*5* *recD1903*::Tn*10*	SMR2601 x P1(SMR2601); Tet^r; RecD$^-$

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3139	RSH469	FC40 *lexA3 malB*::Tn9 *recAo281 srl-300*::Tn*10* *recD6001*::*Tn10kan*	SMR845 x P1(SMR692); Tet^{r}; RecD^{-}
3140	RSH470	FC40 *recAo281 srl-300*::Tn*10* *recD6001*::*Tn10kan*	SMR846 x P1(SMR692); Tet^{r}; RecD^{-}
3149	KIM7	FC40 *Δ(xseA-guaB)* *zff-3139*::Tn*10kan* *ΔxonA300*::*CAT recJ284*::Tn*10*	SMR3011 x P1(SMR690); Tet^{r}; Cam^{r}, Kan^{r},slightly-moderately UV^{s}
3151	RSH471	FC36 *zid-3162*::Tn*10kan* Str^{r}	Spontaneous Str^{r} isolate of SMR3115
3152	RSH472	FC40 *priA2*::*kan spa-116*	Spontaneous LBH^{r} derivative of SMR1489; **NOTE:** the mutation conferring LBH^{r} was named *spa-116*, for suppressor of *priA* and maped to *dnaC*
3153	RSH473	FC40 *priA2*::*kan spa-132*	Spontaneous LBH^{r} derivative of SMR1489; **NOTE:** the mutation conferring LBH^{r} was named *spa-132*, for suppressor of *priA* and maped to *dnaC*
3154	RSH474	FC40 *priA2*::*kan spa-116* *zjj-202*::Tn*10*	SMR3152 x P1(SMR3132); Tet^{r}; LBH^{r}
3155	RSH475	FC40 *priA2*::*kan spa-132* *zjj-202*::Tn*10*	SMR3153 x P1(SMR3132); Tet^{r}; LBH^{r}
3156	RSH476	FC40 *zjj-202*::Tn*10*	SMR506 x P1(SMR3132); Tet^{r}; LBH^{r}
3157	RSH477	FC40 [pET3cY1]	SMR506 transformed with [pET3cY1]; Amp^{r}
3158	RSH478	FC40 [pET3c-priA-K230R]	SMR506 transformed with [pET3c-priA-K230R]; Amp^{r}
3159	RSH479	FC40 [pHM7203]	SMR506 transformed with [pHM7203]; Amp^{r}

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3160	RSH480	FC29 [pET3cY1]	SMR504 transformed with [pET3cY1]; Amp^r
3161	RSH481	FC29 [pET3c-priA-K230R]	SMR504 transformed with [pET3c-priA-K230R]; Amp^r
3162	RSH482	FC29 [pHM7203]	SMR504 transformed with [pHM7203]; Amp^r
3163	RSH483	FC36 *zid-3162*::Tn*10kan* [pET3cY1]	SMR3115 transformed with [pET3cY1]; Amp^r; Pro$^-$
3164	RSH484	FC36 *zid-3162*::Tn*10kan* [pET3c-priA-K230R]	SMR3115 transformed with [pET3c-priA-K230R]; Amp^r; Pro$^-$
3165	RSH485	FC36 *zid-3162*::Tn*10kan* [pHM7203]	SMR3115 transformed with [pHM7203]; Amp^r; Pro$^-$
3166	RSH486	AB1157 *Δ(srlR-recA)304* [pET3cY1]	SMR471 transformed with [pET3cY1]; Amp^r
3167	RSH487	AB1157 *Δ(srlR-recA)304* [pET3c-priA-K230R]	SMR471 transformed with [pET3c-priA-K230R]; Amp^r
3168	RSH488	AB1157 *Δ(srlR-recA)304* [pHM7203]	SMR471 transformed with [pHM7203]; Amp^r
3169	RSH489	FC40 *priA2*::*kan* *dnaC809* *zjj-202*::Tn*10*	SMR1489 x P1(SMR3130); Tet^r; LBH^r
3170	RSH490	FC29 *priA2*::*kan* [pET3cY1]	SMR3133 transformed with [pET3cY1]; Amp^r; LBH^r
3171	RSH491	FC29 *priA2*::*kan* [pET3c-priA-K230R]	SMR3133 transformed with [pET3c-priA-K230R]; Amp^r; LBH^r
3172	RSH492	FC29 *priA2*::*kan* [pHM7203]	SMR3133 transformed with [pHM7203]; Amp^r; LBH^s
3173	RSH493	FC40 *priA2*::*kan* [pET3cY1]	SMR1489 transformed with [pET3cY1]; Amp^r; LBH^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3174	RSH494	FC40 *priA2*::*kan* [pET3c-priA-K230R]	SMR1489 transformed with [pET3c-priA-K230R]; Amp^r; LBH^r
3175	RSH495	FC40 *priA2*::*kan* [pHM7203]	SMR1489 transformed with [pHM7203]; Amp^r; LBH^s
3176	RSH496	FC36 *priA2*::*kan* [pET3cY1]	SMR3135 transformed with [pET3cY1]; Amp^r; Pro$^-$, LBH^r
3177	RSH497	FC36 *priA2*::*kan* [pET3c-priA-K230R]	SMR3135 transformed with [pET3c-priA-K230R]; Amp^r; Pro$^-$, LBH^r
3178	RSH498	FC36 *priA2*::*kan* [pHM7203]	SMR3135 transformed with [pHM7203]; Amp^r; Pro$^-$, LBH^s
3179	RSH499	FC40 *recJ*::Tn*10kan*	SMR1953 cured of prophage by streaking on LBH + EDTA and incubating at 43°C; Kan^r, Tet^s, Str^s, λ^s, λi21^s
3180	RSH500	FC40 *recJ*::Tn*10str*	SMR1953 cured of prophage by streaking on LBH + EDTA and incubating at 43°C; Str^r, Tet^s, Kan^s, λ^s, λi21^s
3181 to 3186	RSH501 to RSH506	FC40 *recD1014* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 1-6 of SMR3109 (Harris *et al.*, 1997b; CHAPTER 6)
3187 to 3191	RSH507 to RSH511	FC40 *recD1014* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 1-5 of SMR3110 (Harris *et al.*, 1997b; CHAPTER 6)
3192 to 3197	RSH512 to RSH517	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 1-6 of SMR3111 (Harris *et al.*, 1997b; CHAPTER 6)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3198 to 3203	RSH518 to RSH523	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 1-6 of SMR3112 (Harris *et al.*, 1997b; CHAPTER 6)
3215	RSH524	FC40 *dnaC809 zjj-202*::Tn*10* *priA2*::*kan*	SMR3247 x P1(SMR840); Kan^r; LBH^r
3216 to 3221	RSH525 to RSH530	FC40 *recD1014* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 7-12 of SMR3109 (Harris *et al.*, 1997b; CHAPTER 6)
3222 to 3326	RSH531 to RSH535	FC40 *recD1014* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 6-10 of SMR3110 (Harris *et al.*, 1997b; CHAPTER 6)
3227 to 3232	RSH536 to RSH541	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 7-12 of SMR3111 (Harris *et al.*, 1997b; CHAPTER 6)
3233 to 3238	RSH542 to RSH547	FC40 *recD1014* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 7-12 of SMR3112 (Harris *et al.*, 1997b; CHAPTER 6)
3247	RSH548	FC40 *dnaC809 zjj-202*::Tn*10*	SMR506 x P1(SMR3130); Tet^r; Kan^s; confirmed *dnaC809* by crossing in *priA2*::*kan* (see SMR3215)
3248	RSH549	FC40 [pET3cY1::*priA1*::*kan* (?)]	SMR3157 x P1(SMR3046); Kan^r & Amp^r; Rif^r, LBH^r; **NOTE:** not sure if the kan insertion is in the chromosome or plasmid as this strain retained the linked marker encoding Rif^r - see SMR3266 for a *bona fide* chromosomal *priA* mutant

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3249	RSH550	FC40 [pET3cY1::*priA2*::*kan* (?)]	SMR3157 x P1(SMR840); Kan^{r} & Amp^{r}; Rif^{r}, LBH^{r}; **NOTE:** not sure if the kan insertion is in the chromosome or plasmid as this strain retained the linked marker encoding Rif^{r} - see SMR3267 for a *bona fide* chromosomal *priA* mutant
3250	RSH551	FC40 [pET3c-priA-K230R::*priA1*::*kan* (?)]	SMR3158 x P1(SMR3046); Kan^{r} & Amp^{r}; Rif^{r}, LBH^{r}; **NOTE:** not sure if the kan insertion is in the chromosome or plasmid as this strain retained the linked marker encoding Rif^{r} - see SMR3268 for a *bona fide* chromosomal *priA* mutant
3251	RSH552	FC40 [pET3c-priA-K230R::*priA2*::*kan* (?)]	SMR3158 x P1(SMR840); Kan^{r} & Amp^{r}; Rif^{r}, LBH^{r}; **NOTE:** not sure if the kan insertion is in chromosome or plasmid as this strain retained the linked marker encoding Rif^{r} - see SMR3269 for a *bona fide* chromosomal *priA* mutant
3252	RSH553	MG1655 *recG162* *zib*::Tn*10*::λTSK	SMR3123 lysogenized with λTSK; Kan^{r}; Str^{r}, Tet^{s}, λ^{r}, λi21^{s}; **NOTE:** grow at <32°C
3253	RSH554	FC40 *recD1014* [pSL5]	SMR3072 transformed with [pSL5]; Kan^{r}
3254	RSH555	FC40 *recD1014* [pSL6]	SMR3072 transformed with [pSL6]; Kan^{r}, Amp^{r}
3255	RSH556	FC40 *priA1*::*kan* Rif^{s} [pET3cY1]	SMR3157 x P1(SMR3046); Kan^{r} & Amp^{r}; Rif^{s}, LBH^{r}

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3256	RSH557	FC40 *priA2*::*kan* Rif^s [pET3cY1]	SMR3157 x P1(SMR840); Kan^r & Amp^r; Rif^s, LBH^r
3257	RSH558	FC40 *priA1*::*kan* Rif^s [pET3c-priA-K230R]	SMR3158 x P1(SMR3046); Kan^r & Amp^r; Rif^s, LBH^r
3258	RSH559	FC40 *priA2*::*kan* Rif^s [pET3c-priA-K230R]	SMR3158 x P1(SMR840); Kan^r & Amp^r; Rif^s, LBH^r
3259	KIM8	FC40 *lexA3 recJ*::Tn*10Str*	SMR868 x P1(SMR3180); Str^r
3260	KIM9	FC40 *lexA3 ΔxonA300*::*CAT*	SMR868 x P1(SMR839); Cam^r; **NOTE:** identical to SMR3466 & SMR3614 (different P1 donors)
3261	RSH560	MG1655 *recG162 zib*::Tn*10str*	SMR3252 cured of prophage by streaking on LBH and incubating at 43°C; Str^r, Kan^s, Tet^s, λ^s, λi21^s
3262	RSH561	FC40 *priA1*::*kan* Rif^s [pHM7203]	SMR3159 x P1(SMR3046); Kan^r & Amp^r; Rif^s, LBH^s
3264	RSH562	FC40 *zib*::Tn*10str*	SMR506 x P1(SMR3261); Str^r; UV^r
3265	RSH563	FC40 *recG162 zib*::Tn*10str*	SMR506 x P1(SMR3261); Str^r; slightly UV^s; *recG* confirmed by crossing in *ruvA*::Tn*10* (see SMR3280)
3266	RSH564	FC40 *priA1*::*kan* Rif^r [pET3cY1]	Spontaneous Rif^r isolate of SMR3255
3267	RSH565	FC40 *priA2*::*kan* Rif^r [pET3cY1]	Spontaneous Rif^r isolate of SMR3256; **NOTE:** identical to SMR3173, but *priA2* moved in last
3268	RSH566	FC40 *priA1*::*kan* Rif^r [pET3c-priA-K230R]	Spontaneous Rif^r isolate of SMR3257

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3269	RSH567	FC40 *priA2::kan* Rif^r [pET3c-priA-K230R]	Spontaneous Rif^r isolate of SMR3258; **NOTE:** identical to SMR3174, but *priA2* moved in last
3270	RSH568	FC40 *priA1::kan* Rif^r [pHM7203]	Spontaneous Rif^r isolate of SMR3262
3271	RSH569	FC40 *recG162 zib-636*::Tn*10*	SMR506 x P1(SMR3123); Tet^r; slightly UV^s
3272	RSH570	FC40 *zib-636*::Tn*10*	SMR506 x P1(SMR3123); Tet^r; UV^r
3274	RDK2821	*thr leuB6 thi thyA trpC1117 hsrk12 hsmk12 str recA13* [pRDK201] [pRG1]	R.D. Kolodner, Dana-Farber Cancer Institute, Boston, MA (Griffin IV, *et al.*, 1990)
3275	RDK1466	*his-4 argE3 leuB6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 kdgK51 supE44 tsx-33 Δ(srlR-recA)304* [pRDK35]	"
3276	RDK1988	*thr leuB6 thi thyA trpC1117 hsrK12 hsmK12 str recA13* [pRG1]	"
3277	RDK1400	*thr leuB6 thi thyA trpC1117 hsrk12 hsmk12 str recA13*	"
3279	RSH571	FC40 *recG162 zib*::Tn*10str* *Δ(recA-srlR)306::Tn10*	SMR3265 x P1(SMR624); Tet^r; extremely UV^s
3280	RSH572	FC40 *recG162 zib*::Tn*10str* *ruvA59*::Tn*10*	SMR3265 x P1(SMR603); Tet^r; extremely UV^s
3292	RSH573	FC40 *recG162 zib*::Tn*10str* [pSL4]	SMR3265 transformed with [pSL4]; Kan^r; Amp^r
3293	RSH574	FC40 *recG162 zib*::Tn*10str* [pSL5]	SMR3265 transformed with [pSL5]; Kan^r
3294	RSH575	FC40 *recG162 zib*::Tn*10str* [pSL6]	SMR3265 transformed with [pSL6]; Kan^r; Amp^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3295	RSH576	FC40 *recG162 zib*::Tn*10str* [pSL7]	SMR3265 transformed with [pSL7]; Kan^r; Tet^r
3296	RSH577	FC40 *recG162 zib*::Tn*10str* *Δ(recA-srlR)306*::*Tn10* [pSL4]	SMR3279 transformed with [pSL4]; Kan^r; Amp^r, Tet^r, extremely UV^s
3297	RSH578	FC40 *recG162 zib*::Tn*10str* *Δ(recA-srlR)306*::*Tn10* [pSL7]	SMR3279 transformed with [pSL7]; Kan^r; Amp^r, Tet^r, extremely UV^s
3298	RSH579	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10* [pSL4]	SMR3090 transformed with [pSL4]; Kan^r; Amp^r, Cam^r, Tet^r
3299	RSH580	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10* [pSL7]	SMR3090 transformed with [pSL7]; Kan^r; Cam^r, Tet^r
3309	RSH581	FC40 *zae*::Tn*10d*-Cam *dnaE915* *zae*::Tn*10*::λTSK	SMR2025 lysogenized with λTSK; Kan^r; Str^r, Tet^s, λ^r, λi21^s; **NOTE:** grow at <32°C
3310	RSH582	FC40 *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae*::Tn*10*::λTSK	SMR2026 lysogenized with λTSK; Kan^r; Str^r, Tet^s, λ^r, λi21^s; **NOTE:** grow at <32°C
3311 to 3314	RSH616 to RSH619	FC40 Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 4-14, 16, 26, 37 of SMR1790 (Harris *et al.*, 1997b; CHAPTER 6)
3315 to 3318	RSH620 to RSH623	FC40 Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 7-1, 20, 21, 39 of SMR1791 (Harris *et al.*, 1997b; CHAPTER 6)
3319 to 3322	RSH624 to RSH627	FC40 *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 4A-15, 17, 24, 28 of SMR3009 (Harris *et al.*, 1997b; CHAPTER 6)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3323 to 3326	RSH628 to RSH631	FC40 *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 7A-4, 7, 8, 22 of SMR3010 (Harris *et al.*, 1997b; CHAPTER 6)
3327 to 3339	RSH632 to RSH644	FC40 *recG162 zib*::Tn*10str* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 1-13 of SMR3292
3340 to 3352	RSH645 to RSH657	FC40 *recG162 zib*::Tn*10str* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 1-13 of SMR3295
3353 to 3365	RSH658 to RSH670	FC40 *recG162 zib*::Tn*10str* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL4]	Spontaneous Lac$^+$ isolates 1-13 of SMR3296
3366 to 3378	RSH671 to RSH683	FC40 *recG162 zib*::Tn*10str* *Δ(recA-srlR)306*::*Tn10* Lac$^+$ [pSL7]	Spontaneous Lac$^+$ isolates 1-13 of SMR3297
3402	RSH583	FC40 *zae*::Tn*10d*-Cam *dnaE915 zae*::Tn*10Str*	SMR3309 cured of prophage by streaking on LBH and incubating at 43°C; Str^r, Kan^s, Tet^s, λ^s, $\lambda i21^s$
3403	RSH584	FC40 *zae*::Tn*10d*-Cam *dnaE*$^+$ *zae*::Tn*10Str*	SMR3310 cured of prophage by streaking on LBH and incubating at 43°C; Str^r, Kan^s, Tet^s, λ^s, $\lambda i21^s$
3404	RSH585	FC40 *mutL211*::Tn*5*	SMR506 x P1(SMR91); Kan^r; mutator (Lac$^+$); **NOTE:** identical to SMR3405 & 3428 (different P1 donors) (Harris *et al.*, 1997c; APPENDIX IV)
3405	RSH586	FC40 *mutL211*::Tn*5*	SMR506 x P1(SMR346); Kan^r; mutator (Lac$^+$); **NOTE:** identical to SMR3404 & 3428 (different P1 donors)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3406	RSH587	FC40 *mutS201*::Tn*5*	SMR506 x P1(SMR438); Kan^r; mutator (Lac^+); **NOTE:** identical to SMR3407 (different P1 donor) [(Harris *et al.*, 1997a) & CHAPTER 4]
3407	RSH588	FC40 *mutS201*::Tn*5*	SMR506 x P1(SMR390); Kan^r; mutator (Lac^+); **NOTE:** identical to SMR3406 (different P1 donor)
3409	NR3835 polA1	*polA1 zgj-203*::Tn*10 Δ(pro-lac) ara thi trpE9777* [F *pro*$^+$ *lac*$^+$]	M. Radman, Paris; Tn linked to *polA*
3424	RSH589	FC40 *mutL211*::Tn5 *Δ(recA-srlR)306::Tn10*	SMR3404 x P1(SMR624); Tet^r; extremely UV^s, mutator (Lac^+); **NOTE:** identical to SMR3425 & SMR3429 (different P1 recipients)
3425	RSH590	FC40 *mutL211*::Tn5 *Δ(recA-srlR)306::Tn10*	SMR3405 x P1(SMR624); Tet^r; extremely UV^s, mutator (Lac^+); **NOTE:** identical to SMR3424 & SMR3429 (different P1 recipients)
3426	RSH591	FC40 *mutS201*::Tn*5* *Δ(recA-srlR)306::Tn10*	SMR3406 x P1(SMR624); Tet^r; extremely UV^s, mutator (Lac^+); **NOTE:** identical to SMR3427 (different P1 recipient) (Harris *et al.*, 1997a; CHAPTER 4)
3427	RSH592	FC40 *mutS201*::Tn*5* *Δ(recA-srlR)306::Tn10*	SMR3407 x P1(SMR624); Tet^r; extremely UV^s, mutator (Lac^+); **NOTE:** identical to SMR3426 (different P1 recipient)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3428	RSH593	FC40 *mutL211*::Tn*5*	SMR506 x P1(SMR620); Kan^{r}; strong mutator (Lac^{+}); **NOTE:** identical to SMR3404 & 3405 (different P1 donors) (Harris *et al.*, 1997a; CHAPTER 4)
3429	RSH594	FC40 *mutL211*::Tn*5* *Δ(recA-srlR)306::Tn10*	SMR3428 x P1(SMR624); Tet^{r}; extremely UV^{s}, mutator (Lac^{+}); **NOTE:** identical to SMR3424 & SMR3425 (different P1 recipients) (Harris *et al.*, 1997a; CHAPTER 4)
3441	RS1	*polA12*(Ts) *rha lac ilv* *fadAB3165*::Tn*10kan*	SMR160 x P1(SMR3074); Kan^{r}; *polA12*(Ts) confirmed by crossing in *recA* (see SMR3456)
3442	RS2	*polA*$^{+}$ *fadAB3165*::Tn*10kan* *rha lac ilv*	SMR160 x P1(SMR3074); Kan^{r}; *polA*$^{+}$ confirmed by crossing in *recA* (see SMR3457)
3443	LN2926	Hfr PR191 Str^{r} *ΔrecBC*::Amp^{r}	J.-M. Louarn, France; **NOTE:** apparently P1-resistant
3444	LN3424	W1485 *thy leu ΔrecD*::Amp^{r}	J.-M. Louarn, France
3445	SO113	*trp lacZ rpsL thi codA5*	J. Neuhard, Copenhagen, Denmark
3453	KIM10	FC40 *lexA3 recJ*::Tn*10Str* *ΔxonA300::CAT*	SMR3259 x P1(SMR3070); Cam^{r}
3454	KIM11	FC40 *mutL211*::Tn*5* *ΔxonA300::CAT*	SMR3428 x P1(SMR3070); Cam^{r}; Kan^{r}
3455	KIM12	FC40 *mutS201*::Tn*5* *ΔxonA300::CAT*	SMR3406 x P1(SMR3070); Cam^{r}; Kan^{r}
3456	RS3	*polA12*(Ts) *rha lac ilv* *fadAB3165*::Tn*10kan* *Δ(recA-srlR)306::Tn10*	SMR3441 x P1(SMR624); Tet^{r}; extremely UV^{s}, Ts (i.e. dead) at 43°C

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3457	RS4	*polA*$^+$ *fadAB3165*::Tn*10kan* *rha lac ilv* Δ*(recA-srlR)306*::*Tn10*	SMR3441 x P1(SMR624); Tet^r; extremely UV^s, Ts$^+$
3463	RSH595	*trp rpsL thi codA5 lac*$^+$	SMR3445 x P1(SMR816); Lac$^+$; 5FC^r, 5FU^s; **NOTE:** control for transductional mapping of mutations that conferred 5-FC^r (Torkelson *et al.*, 1997)
3464	RSH596	*trp rpsL thi codA*$^+$*lac*$^+$	SMR3445 x P1(SMR816); Lac$^+$; 5FC^s, 5FU^s
3465	MJ17	SMR423 Δ*xseA18*::*amp*	Lab collection (Harris *et al.*, 1997c; APPENDIX IV)
3466	KIM13	FC40 *lexA3* Δ*xonA300*::*CAT*	SMR868 x P1(SMR3070); Cam^r; **NOTE:** identical to SMR3260 (different P1 donor) & SMR3614 (constructed independently)
3467	KIM14	FC40 Δ*xonA300*::*CAT* *mutS201*::Tn*5*	SMR3070 x P1(SMR438); Kan^r; Cam^r, mutator (Lac$^+$)
3468	KIM15	FC40 Δ*xonA300*::*CAT* *mutL211*::Tn*5*	SMR3070 x P1(SMR620); Kan^r; Cam^r, mutator (Lac$^+$)
3469	KIM16	FC40 Δ*xonA300*::*CAT* *recJ284*::Tn*10*	SMR3089 x P1(SMR690); Tet^r; Cam^r; **NOTE:** identical to SMR3090 (different P1 donor) & SMR3470 (different P1 recipient)
3470	KIM17	FC40 *xonA300*::*CAT* *recJ284*::Tn*10*	SMR3070 x P1(SMR690); Tet^r; Cam^r; **NOTE:** identical to SMR3090 (different P1 donor) & SMR3469 (different P1 recipient)
3472	MJ18	FC40 Δ*xseA18*::*amp*	Lab collection (Harris *et al.*, 1997c; APPENDIX IV)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3474	RS5	FC36 *fadAB3165*::Tn*10kan*	SMR505 x P1(3441); Kan^r; *polA*$^+$ confirmed by crossing in *recA* (see SMR3476)
3475	RS6	FC36 *polA12*(Ts) *fadAB3165*::Tn*10kan*	SMR505 x P1(3441); Kan^r; *polA12*(Ts) confirmed by crossing in *recA* (see SMR3477)
3476	RS7	FC36 *fadAB3165*::Tn*10kan* *Δ(recA-srlR)306::Tn10*	SMR3474 x P1(SMR624); Tet^r; extremely UV^s, Ts$^+$
3477	RS8	FC36 *polA12*(Ts) *fadAB3165*::Tn*10kan* *Δ(recA-srlR)306::Tn10*	SMR3475 x P1(SMR624); Tet^r; extremely UV^s, Ts (i.e. dead) at 43°C
3478	KIM18	FC40 *recJ284*::Tn*10* *ΔxseA18::amp*	SMR690 x P1(SMR3472); Amp^r; Tet^r
3479	KIM19	FC40 *ΔxonA300::CAT* *ΔxseA18::amp*	SMR3070 x P1(SMR3472); Amp^r; Cam^r
3480	KIM20	FC40 *recJ*::Tn*10str* *ΔxseA18::amp*	SMR3180 x P1(SMR3472); Amp^r; Str^r
3481	KIM21	FC40 *ΔxonA300::CAT* *recJ284*::Tn*10* *ΔxseA18::amp*	SMR3469 x P1(SMR3472); Amp^r; Cam^r, Tet^r; **NOTE:** identical to SMR3482 & 3485 (different P1 recipients) (Harris *et al.*, 1997c; APPENDIX IV)
3482	KIM22	FC40 *ΔxonA300::CAT* *recJ284*::Tn*10* *ΔxseA18::amp*	SMR3470 x P1(SMR3472); Amp^r; Cam^r, Tet^r; **NOTE:** identical to SMR3481 & 3485 (different P1 recipients)
3483	KIM23	FC40 *recD1014* *ΔxonA300::CAT*	SMR3072 x P1(SMR3070); Cam^r
3484	KIM24	FC40 *lexA3 recJ*::Tn*10str* *ΔxseA18::amp*	SMR3259 x P1(SMR3472); Amp^r; Str^r

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3485	KIM25	FC40 *ΔxonA300*::*CAT recJ284*::Tn*10 ΔxseA18*::*amp*	SMR3090 x P1(SMR3472); Amp^r; Cam^r, Tet^r; **NOTE:** identical to SMR3481 & 3482 (different P1 recipients)
3486	KIM26	FC40 *lexA3 ΔxonA300*::*CAT ΔxseA18*::*amp*	SMR3466 x P1(SMR3472); Amp^r; Cam^r; **NOTE:** identical to 3487 (different P1 recipient)
3487	KIM27	FC40 *lexA3 ΔxonA300*::*CAT ΔxseA18*::*amp*	SMR3614 x P1(SMR3472); Amp^r; Cam^r; **NOTE:** identical to 3486 (different P1 recipient)
3488	KIM28	JC11450 *ΔxonA300*::*CAT recJ284*::Tn*10 ΔxseA18*::*amp*	SMR1403 x P1(3472); Amp^r; Cam^r, Tet^r (Harris *et al*., 1997c; APPENDIX IV)
3490	RS9	FC36 *fadAB3165*::Tn*10kan* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated 3474 x SMR506; Pro$^+$ & Kan^r at 30°C; Lac$^-$ (revertable)
3491	RS10	FC36 *polA12*(Ts) *fadAB3165*::Tn*10kan* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated 3475 x SMR506; Pro$^+$ & Kan^r at 30°C; Lac$^-$ (revertable)
3492	RS11	FC36 *fadAB3165*::Tn*10kan Δ(recA-srlR)306*::*Tn10* [F'*proAB*$^+$ *lacI33ΩlacZ*]	Mated 3476 x SMR506; Pro$^+$ & Kan^r at 30°C; Lac$^-$ (revertable)
3514	RSH597	FC29 *mutL218*::Tn*10*	SMR504 x P1(SMR308); Tet^r; mutator (Nal^r & Str^r)
3520	RSH598	FC29 *mutL218*::Tn*10* [pSL4]	SMR3514 transformed with [pSL4]; Kan^r; Amp^r, Tet^r, mutator (Nal^r & Str^r)
3521	RSH599	FC29 *mutL218*::Tn*10* [pSL6]	SMR3514 transformed with [pSL6]; Kan^r; Amp^r, Tet^r, mutator (Nal^r & Str^r)

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3524	KIM29	JC11450 *mutL211*::Tn*5*	SMR122 x P1(SMR91); Kan^r; mutator (Nal^r & Arg$^+$) (Harris *et al.*, 1997c; APPENDIX IV)
3550	KIM30	FC40 *recJ*::Tn*10str* *ΔxseA18*::*amp* *ΔxonA300*::*CAT*	SMR3480 x P1(SMR3070); Cam^r; Amp^r, Str^r
3551	KIM31	FC40 *lexA3 recJ*::Tn*10str* *ΔxonA300*::*CAT ΔxseA18*::*amp*	SMR3453 x P1(SMR3472); Amp^r; Str^r, Cam^r
3552	KIM32	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10 ΔxseA18*::*amp* *mutL211*::Tn*5*	SMR3481 x P1(SMR620; Kan^r; Amp^r, Tet^r, Cam^r; mutator (Lac$^+$)
3554 to 3565	RSH600 to RSH611	FC40 Lac$^+$ [pSL5]	Spontaneous Lac$^+$ isolates 1-12 of SMR1713
3566	KIM33	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10 ΔxseA18*::*amp* [pSL4]	SMR3481 transformed with pSL4 ; Kan^r
3567	KIM34	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10 ΔxseA18*::*amp* [pSL5]	SMR3481 transformed with pSL5; Kan^r
3568	KIM35	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10 ΔxseA18*::*Amp* [pSL6]	SMR3481 transformed with pSL6; Kan^r
3569	KIM36	FC40 *ΔxonA300*::*CAT* *recJ284*::Tn*10 ΔxseA18*::*amp* [pSL7]	SMR3481 transformed with pSL7; Kan^r
3572	KIM37	FC40 *recJ*::Tn*10Str* *ΔxseA18*::*amp* *ΔxonA300*::*CAT* *Δ(recA-srlR)306*::*Tn10*	SMR3550 x P1(SMR624); Tet^r; Cam^r, Amp^r, Str^r; **NOTE:** UV^s apparently indistinguishable from either parent
3587	RSH612	AB1157 *ΔxseA18*::*amp*	SMR752 x P1(SMR3481); Amp^r; more sensitive to 2μg/ml nalidixic acid in LBH than SMR752

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3590	RSH613	JC11450 *ΔrecD*::Amp^{r}	SMR122 x P1(SMR3444); Amp^{r}; UV^{r}, RecD^{-}
3598	RSH614	FC40 *zff-3139*::Tn*10kan*	SMR506 x P1(SMR758); Kan^{r}; Gua^{+}
3599	RSH615	AB1157 *zff-3139*::Tn*10kan*	SMR752 x P1(SMR758); Kan^{r}; Gua^{+}
3614	KIM38	FC40 *lexA3 ΔxonA300*::*CAT*	SMR868 x P1(SMR3070); Cam^{r}; **NOTE:** identical to SMR3260 (different P1 donor) & SMR3466 (constructed independently)
3615 to 3626	KIM39 to KIM50	FC40 Lac^{+}	Spontaneous Lac^{+} isolates 1-12 of SMR506
3627 to 3638	KIM51 to KIM62	FC40 *xonA300*::*CAT recJ284*::Tn*10 ΔxseA18*::*amp* Lac^{+}	Spontaneous Lac^{+} isolates 1-12 of SMR3481
3640	RSH684	MG1655 *ΔdnaQ903*::*tet spq-2 zae*::Tn*10d*-Cam	SMR1306 x P1(SMR540); Cam^{r}; Ts^{+}; **NOTE:** all isolates (40/40) were Ts^{+} (presumably this is because *spq-2* is needed for a cell to tolerate *ΔdnaQ*)
3657	SH2101	*leu ara* Δ(*pro-lac*) *thi* Sm^{r} *polBΔ1*::ΩSm-Sp (small-size colony)	M. Goodman, University of Southern California *via* L. Reha Kranz (Escarcellar *et al.*, 1994)
3658	RSH685	FC40 *polBΔ1*::ΩSm-Sp *leu*	SMR506 x P1(SMR3657); Spc^{r}; Leu^{-}; **NOTE:** small colony isolate
3659	RSH686	FC40 *polBΔ1*::ΩSm-Sp *leu*	SMR506 x P1(SMR3657); Spc^{r}; Leu^{-}; **NOTE:** large colony isolate, probably due to a growth defect-suppressor

TABLE I-1. contd.:

SMR number	Strain name	Relevant genotype	Construction information, source, reference &/or note
3660	RSH687	FC40 *polBΔ1*::ΩSm-Sp *leu*	SMR506 x P1(SMR3658); Spc^r; Leu$^-$; **NOTE:** small colony isolate
3661	RSH688	FC40 *polBΔ1*::ΩSm-Sp	SMR506 x P1(SMR3658); Spc^r; Leu$^+$; **NOTE:** small colony isolate
3662	RSH689	FC40 *polBΔ1*::ΩSm-Sp	SMR506 x P1(SMR3659); Spc^r; Leu$^+$; **NOTE:** small colony isolate
3663	RSH690	FC40 *polBΔ1*::ΩSm-Sp *leu*	SMR506 x P1(SMR3659); Spc^r; Leu$^-$; **NOTE:** small colony isolate

TABLE I-2. Plasmids.

Plasmid	Construction information, source, and/or reference
pAL5	Contains the herpes simplex virus TK1 gene and a TK1 gene fragment aligned directly, but separated by 1397bp; for assaying homologous recombination in mice or *E. coli*; encodes Kan^r (=Neo^r); A. Waldman, Yale University (Waldman and Liskay, 1987) (see SMR576)
pBLW20	pET21d-derived plasmid which overproduces RecF under *tac* promoter control; encodes LacI^q; Amp^r; M. Cox, University of Wisconson, Madison (Webb *et al.*, 1995); **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR3004, SMR3060, SMR3062 & SMR3064)
pCAT19	Carries the chloramphenicol acetyl transferase gene (CAT19) flanked by polylinkers; Cam^r; J. Elliott, University of Alberta, Edmonton, (Fuqua, 1992) (see SMR688)
pDNAE OPIΔ*Pst*I-*Apa*LI	Overproduces the alpha subunit of the PolIII holoenzyme (*dnaE*) under *tac* promoter control; encodes LacI^q; Amp^r; C. McHenry; University of Colorado, Denver; **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR2078 & SMR2209)
pET21d	Cloning vector; encodes LacI^q; Amp^r; Novagen, Madison *via* M. Cox, University of Wisconson, Madison; **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR3005, SMR3061, SMR3063 & SMR3065)
pET3c-priA-K230R	Site-directed mutant of pET3cY1; overproduces PriAK230R which lacks helicase activity but still catalyzes primosome assembly; Amp^r; K. Marians, Memorial Sloan-Kettering Cancer Institute, New York (Zavitz and Marians, 1992) (see SMR3158, SMR3161, SMR3164, SMR3167, SMR3171, SMR3174 & SMR3177)
pET3cY1	pBR322-derived plasmid which overproduces the primosome assembly protein PriA (*priA*); Amp^r; K. Marians, Memorial Sloan-Kettering Cancer Institute, New York (Zavitz and Marians, 1992) (see SMR3157, SMR3160, SMR3163, SMR3166, SMR3170, SMR3173 & SMR3176)
pHC5	Overproduces DNA PolII (*polB*) under *tac* promoter control; encodes LacI^q; Amp^r; R. Moses, Oregon Health Sciences University; **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR2079 & SMR2081)

TABLE I-2. contd.:

Plasmid	Construction information, source, and/or reference
pHC5Δ*Hinc*II	pHC5-derived plasmid which encodes null allele of DNA PolII (*polB*) under *tac* promoter control; encodes LacI^q; Amp^r; R. Moses, Oregon Health Sciences University; **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR2080 & SMR2082)
pHM7203	pBR322-derived plasmid which is able to replicate in a *priA* mutant; Amp^r &Tet^r; H. Masai, Tokyo (see SMR3159, SMR3162, SMR3165, SMR3168, SMR3172, SMR3175 & SMR3178)
pHN3	Overproduces the alpha (*dnaE*) and epsilon (*dnaQ*) subunits of the PolIII holoenzyme under *tac* promoter control; encodes LacI^q; Amp^r; C. McHenry, University of Colorado, Denver; **NOTE:** *lac* homology (*lacI^q*) stimulates reversion of the *lac* frameshift mutation (see SMR2077 & SMR2208)
pHSG415	Low-copy, pSC101-derived cloning vector; Cam^r; S. Sedgwick, MRC, London (Spanos and Sedgwick, 1984) (see SMR1634)
pMH101	Low-copy, pHSG415-derived plasmid which overproduces DNA polymerase I (*polA*); Cam^r; S. Sedgwick, MRC, London (Spanos and Sedgwick, 1984) (see SMR1633)
pOXTc	Mini-F plasmid used for quantitative conjugations; Tet^r; L. Frost, University of Alberta, Edmonton (see SMR3091)
pRDK35	Derivative of pBR322 carrying an oligonucleotide insertion in the *tet* gene; Amp^r, Tet^s; R.D. Kolodner, Dana-Farber Cancer Institute, Boston (Doherty *et al.*, 1983) [see SMR3275 & (Torkelson *et al.*, 1997)]
pRDK201	pBR322-derived plasmid with a new ribosome binding site and *tac* promoter that overproduces RecF when combined with pRG1 & induced by IPTG; Amp^r; R.D. Kolodner, Dana-Farber Cancer Institute, Boston (Griffin IV and Kolodner, 1990) (see SMR3274)
pRG1	Constructed by inserting the *lacI^q* gene into the *Pst*I site of the *bla* gene of pACYC177; Kan^r; R.D. Kolodner, Dana-Farber Cancer Institute, Boston (Griffin IV and Kolodner, 1990); **NOTE:** *lac* homology (*lacI^q*) may stimulate reversion of the *lac* frameshift mutation (see SMR3274 & SMR3276)

TABLE I-2. contd.:

Plasmid	Construction information, source, and/or reference
pRH1	Constructed by ligating an 820bp *Taq*I fragment of pCAT19, which contained the CAT19 gene, into *Cla*I-digested pAL5; the CAT19 cassette was inserted between the TK cassettes such that *Bam*HI cleavage produced fragments of 6.2, 2.7 and 1.5 kb; this was transformed into SMR471, selecting Cam^{r}, to produce SMR735
pRH2	Constructed by ligating an 820bp *Taq*I fragment of pCAT19, which contained the CAT19 gene, into *Cla*I-digested pTK2TK1-8; the CAT19 cassette was inserted between the TK cassettes such that *Bam*HI cleavage produced fragments of 5.8, 2.7 and 1.6 kb; this was transformed into SMR471, selecting Cam^{r}, to produce SMR736
pSL4	Kan^{r} derivative of pBR322; Amp^{r} (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6) (see SMR1790)
pSL5	Derivative of pSL4 which overproduces *E. coli* MutL; Kan^{r} (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6) (see SMR1713)
pSL6	Derivative of pSL4 which overproduces *E. coli* MutS; Kan^{r} & Amp^{r} (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6) (see SMR1747)
pSL7	Derivative of pSL4 which overproduces *E. coli* MutL and MutS; Kan^{r} & Tet^{r}; **NOTE:** expression of Tet^{r} is poor, therefore, maintain with kanamycin (Harris *et al.*, 1997b; Longerich, 1997; CHAPTER 6) (see SMR1791)
pTK2TK1-8	Contains the herpes simplex virus TK1 gene and a TK2 gene fragment, which is 81% identical to TK1, aligned directly, but separated by 1397bp; for assaying homeologous recombination in mice or *E. coli*; encodes Kan^{r} (=Neo^{r}); A. Waldman, Yale University (Waldman and Liskay, 1987) (see SMR575)
pW17	Derivative of pBR322 with a +1G in the *tet* gene; Amp^{r} & Tet^{s}; G. Maenhaut-Michel, Belgium (Koffel-Schwartz *et al.*, 1984; Torkelson *et al.*, 1997) (see SMR1357 & SMR1401)
pW18	Derivative of pBR322 with a -1G in the *tet* gene; Amp^{r} & Tet^{s}; G. Maenhaut-Michel, Belgium (Koffel-Schwartz *et al.*, 1984; Torkelson *et al.*, 1997) (see SMR1356 & SMR1400)

TABLE I-2. contd.:

Plasmid	Construction information, source, and/or reference
pX2	Derivative of pBR322 with a -1CG in the *tet* gene; Amp^r & Tet^s; G. Maenhaut-Michel, Belgium (Burnhouf and Fuchs, 1985; Torkelson *et al.*, 1997) (see SMR1358 & SMR1402)

TABLE I-3. Abbreviations and symbols.

χ	Chi (Crossover hotspot instigator), GCTGGTGG
λ	Bacteriophage lambda
$\lambda^{r/s}$	λ resistant or sensitive
$\lambda i21^{r/s}$	λimmunity-21 resistant or sensitive
λ p*recA*	λ p*recA* (=SR106 = *cIts857 recA*$^+$) (F. Stahl laboratory collection, Eugene, Oregon); Lysogenization of a *recA* mutant with λ p*recA* facilitates further manipulation of a cell's genes with methods that require RecA to function (*e.g.* transduction). Curing of λ p*recA* renders the cells Rec$^-$ once again (*e.g.* used in Harris *et al.*, 1996 to construct the doubly Rec$^-$ strain, *ruvA recA recG*).
λTSK	λTet-Str-Kan [aka: "tet-blaster" = λSR181 = *cIts857* Tn*10*(*tetA*::*kan* /*tetA*::*str*)] (François *et al.*, 1987); this phage has homology to the *tet* gene of Tn*10*, such that lysogenizing a Tn*10*-bearing strain, selecting Kan^r or Str^r, and screening for Tet^s yields a strain with λTSK integrated in the Tn*10*. Curing this strain of λ by incubation at 42°C in absence of any antibiotic yields 4 possible products: (i) Tn*10kan*, (ii) Tn*10str*, (iii) Tn*10*, or (iv) Tn*10*(Tet^s); **NOTE:** Str^r is only conferred on minimal media (E or M9).
Am	Amber nonsense mutation
Amp$^{r/s}$	Ampicillin resistant or sensitive
Arg$^{+/-}$	Arginine prototroph or auxotroph
AS	Amber mutation suppressor
Cam$^{r/s}$	Chloramphenicol resistant or sensitive
CGSC	*E. coli* Genetic Stock Center, Yale University (see Berlyn, 1996) for a discussion of how to use this database and to obtain strains]
CW	Cindy Wong
Gal$^{+/-}$	Galactose fermentation proficient or deficient
His$^{+/-}$	Histidine prototroph or auxotroph
HR	Haide Razavy
Kan$^{r/s}$	Kanamycin resistant or sensitive
KIM	Kimberly J. Ross
Lac$^{+/-}$	Lactose fermentation proficient or deficient
LBH$^{r/s}$	Rich media (LBH) resistant or sensitive
Leu$^{+/-}$	Leucine prototroph or auxotroph
Mal$^{+/-}$	Maltose fermentation proficient or deficient
MJ	Mary-Jane Lombardo

TABLE I-3. contd.:

Nal$^{r/s}$	Nalidixic acid resistant or sensitive
Oc	Ochre nonsense mutation
Pro$^{+/-}$	Proline prototroph or auxotroph
Pyr$^{+/-}$	Pyrimidine biosynthesis proficient or deficient
R17$^{r/s}$	R17 (a male-specific phage) resistant or sensitive
Rec$^{+/-}$	Recombination proficient or deficient
Rif$^{r/s}$	Rifampicin resistant or sensitive
RS	Roger Sidhu
RSH	Reuben S. Harris
SL	Simonne Longerich
Sp$^{r/s}$	Spectinomycin resistant or sensitive
Spc$^{r/s}$	Spectinomycin resistant or sensitive
SMR	Susan M. Rosenberg
Sm$^{r/s}$	Streptomycin resistant or sensitive
Srl$^{+/-}$	Sorbitol fermentation proficient or deficient
Str$^{r/s}$	Streptomycin resistant or sensitive
Su	Amber suppressor
SZ	Susan K. Szigety
T7$^{r/s}$	T7 (a female-specific phage) resistant or sensitive
Tet$^{r/s}$	Tetracycline resistant or sensitive
Thr$^{+/-}$	Proline prototroph or auxotroph
Tn	Transposon
Trp$^{+/-}$	Tryptophan prototroph or auxotroph
Ts	Temperature sensitive
Ts^{+}	Temperature resistant
UV$^{r/s}$	Ultraviolet light resistant or sensitive

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APPENDIX II

SOS IN ADAPTIVE MUTATION

INTRODUCTION

E. coli responds to DNA damage or to an inhibition of DNA replication by inducing the SOS response (reviewed by Witkin, 1991; Walker, 1996). The SOS response includes de-repression of more than 20 genes controlled by LexA, whose gene products function in DNA repair, DNA replication, recombination, cell cycle inhibition, and notably, mutation.

Induction of the LexA regulon (FIGURE II-1) is thought to begin with the exposure of single-strand DNA (ssDNA). ssDNA is proposed to activate the latent coprotease activity of RecA (Higashitani *et al.*, 1995; but see Witkin, 1991). Activated RecA (RecA*), promotes the cleavage of the LexA repressor. This releases LexA from the operators of genes of the LexA regulon resulting in their de-repression (FIGURE II-1). Many of these genes, including *lexA* and *recA*, are also expressed constitutively at a lower level. RecA* also promotes the cleavage of the repressors of the bacteriophages λ, P22, 434, and ϕ80, and the cleavage of UmuD, a protein that, in its cleaved form (UmuD'), promotes SOS-associated mutagenesis *via* error prone DNA synthesis (Murli and Walker, 1993).

The SOS response has been detected recently in old, starving *E. coli* colonies (Taddei *et al.*, 1995). To assay SOS induction in colonies, Taddei *et al.* (1995) monitored cleavage of the λ cI repressor *via* an epigenetic switch (Toman *et al.*, 1985). In this elegant assay system, the λ *cro* gene is fused to the *gal* operon and both are repressed tightly by cI, rendering the cells Gal$^-$ and the colonies white on indicator plates. However, if SOS is induced in these cells, cI cleavage allows expression of the *cro-gal* fusion. Newly synthesized Cro causes a heritable, irreversible switch by repressing *cI*. This results in red colony color on indicator plates. Taddei *et al.* found that switching in old colonies depends on *recA*. *recA430*, which encodes a recombination-proficient, proteolytically-inactive RecA protein (Devoret *et al.*, 1983; Roca and Cox, 1990), blocks the switch. Thus, cI is inactivated in aged, starved colonies *via* a genuine RecA*-dependent SOS response. The color change was impaired in a *lexA1* mutant, which encodes a repressor protein resistant RecA*-mediated cleavage. This implies that a component of the SOS regulon is required at de-repressed levels for the switch to occur. The inhibition caused by *lexA1* was overcome by RecA overproduction using the operator-constitutive allele *recAo98*. Therefore, the only component of the SOS regulon needed for the switch at de-repressed levels is RecA. That the signal triggering SOS is nutritional is indicated by their finding that cyclic AMP (catabolite repression) is necessary for starvation-induced SOS. These results indicate that nutrient deprived *E. coli* experience an SOS response and that cyclic AMP is part of the mechanism for transducing signals from the environment to their DNA.

Because the SOS response is a key modifier of DNA metabolism (Witkin, 1991; Walker, 1996) and it occurs in starved *E. coli* (Taddei *et al.*, 1995), it is an excellent candidate for a component of adaptive mutagenesis. We investigated the role of SOS in Rec-dependent reversion in the *lac* frameshift assay system (see CHAPTER 1).

RESULTS AND DISCUSSION

De-repression of the LexA Regulon Is Necessary for Lac$^+$ Adaptive Mutation Cairns and Foster found evidence for a role for SOS induction in adaptive reversion of the *lac* frameshift mutation (Cairns and Foster, 1991). The *lexA3*[1] allele, which encodes an uncleavable repressor, decreased reversion about 3-fold. This was interpreted correctly to mean that one, or more, of the proteins repressed by LexA is required for full levels of adaptive mutation. They also reported that the required protein was RecA itself, as overproduction of RecA using an operator-constitutive allele

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[1] *lexA1* and *lexA3* cause similar, if not identical phenotypes (Mount *et al.*, 1972).

(*recAo281*[2], Volkert *et al.*, 1981) restored the mutability of a *lexA3* strain (Cairns and Foster, 1991). However, attempts to repeat this result were unsuccessful. I[3] and Foster *et al.* (1996) discovered that their *lexA3 recAo281* (Cairns and Foster, 1991) strain was incorrect -- it is *lexA*$^+$. This left open the possibility that de-repression of other LexA-controlled gene(s) might be required for full levels of adaptive mutation.

To test this possibility, a *bona fide lexA3 recAo281* double mutant was constructed and reversion to Lac$^+$ examined. Data in FIGURE II-2-A show that overproduction of RecA by *recAo281* does not affect reversion of a *lexA3* strain (mentioned also by Foster *et al.*, 1996). Furthermore, the inability of *recAo281* to suppress *lexA3* could not be attributed to growth or death as the number of viable frameshift-bearing cells on the experimental plates varied less than 2-fold through the experiment's duration (FIGURE II-2-B). Therefore, de-repression of one or more LexA-repressed genes, other than, or in addition to, RecA is required for full levels of recombination-dependent mutation.

A LexA-repressed gene that is necessary for adaptive mutation could be expected to -- (i) confer adaptive hypomutation similar to or more severe than *lexA3* when absent; and (ii) restore or partially-restore the mutability of a *lexA3* strain when overproduced. However, if de-repressed levels of more than one LexA-regulated gene is required for efficient adaptive mutation, then overcoming the hypomutation caused by *lexA3* could require coordinate overexpression of all these genes. Obvious candidate genes include *ruvA* and *ruvB*, which are both repressed by LexA (Shurvinton and Lloyd, 1982; Benson *et al.*, 1988; Shinagawa *et al.*, 1988; FIGURE II-1), and are also required for recombination-dependent adaptive mutation (CHAPTER 3; Harris *et al.*, 1996; see also Foster *et al.*, 1996). Some other candidate genes include *recF* (see below), *dinB* (encodes a protein implicated in untargeted UV mutagenesis of λ; Brotcorne-Lannoye and Maenhaut-Michel, 1986), *dinP*(encodes a protein similar to UmuC that may also have a role in mutagenesis; Ohmori *et al.*, 1985), *dinF* (see below), and *recN* (encodes a protein implicated in the repair of DNA double-strand breaks; Picksley *et al.*, 1984a; 1984b).

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[2] *recAo98* and *recAo281* are identical base substitution mutations in the operator of *recA* (Clark, 1982; Volkert *et al.*, 1981). LexA is unable to bind to this operator sequence which results in constitutive overproduction of RecA (Clark, 1982; Volkert *et al.*, 1981; Walker, 1996).

[3] The following cross showed that the presumed Cairns and Foster *lexA3 recAo281* double mutant is *lexA*$^+$: transduction of this strain with P1 from a *recA*(Ts) strain, thereby replacing the *recAo281* allele, yielded an *E. coli* strain resistant to UV at the permissive temperature, instead of a strain with the UV sensitivity of a *lexA3* mutant (see SMR897, APPENDIX I).

UmuCD' and UvrABC Are Unlikely Players in Lac$^+$ Adaptive Mutation

Mutagenesis by UV and by a number of chemicals requires UmuCD' to process the DNA damage (Murli and Walker, 1993). *recA*, *umuD*, and *umuC* are essential. RecA* is required for co-cleavage of LexA, which induces expression of all three genes, and to process UmuD to its active form, UmuD'. The UmuC-UmuD' complex is proposed to mediate translesion, mutagenic DNA synthesis, possibly *via* an interaction with DNA polymerase III (reviewed by Walker, 1996).

The possibility that adaptive mutagenesis and SOS mutagenesis occur *via* the same mechanism was excluded by results of Cairns and Foster (Cairns and Foster, 1991). They reported that two mutations that abolish SOS mutagenesis had little effect on adaptive mutation, *recA433*, which encodes a protein unable to process UmuD to UmuD', and a null allele of *umuC* (their data were not shown).

The UvrABC nucleotide excision repair pathway in *E. coli* removes a variety of DNA lesions that block DNA replication (reviewed by Rupp, 1996). *uvrA*, *uvrB*, and *uvrD* are under LexA control (FIGURE II-1). These genes act with *uvrC*[4] in nucleotide excision repair. This DNA repair pathway could be required for adaptive mutation if it promotes initiation of DNA synthesis that gives rise to mutations (Foster, 1993). Alternatively, UvrABC could prevent adaptive mutation if it repairs spontaneous DNA lesions, which, if allowed to persist, would lead to mutations (Foster, 1993). These hypotheses predict that inactivation of this repair pathway would either abolish or elevate adaptive mutation respectively. Neither predication was borne-out as a *uvrA* mutation was reported to have no effect (unfortunately, data were not shown, Foster, 1993). Thus, the UvrABC excision repair pathway appears unlikely to play a role in Lac^+ adaptive mutation[5].

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[4] *uvrC* also may be a member of the SOS regulon (van Sluis *et al.*, 1984). However, van Sluis *et al.* (1984) measured *uvrC* expression from a plasmid derived from pBR322, whose copy number also increases in response to DNA damage. Thus, the apparent inducibility of *uvrC* could be attributed to pBR322 (Walker, 1996).

Identification of the LexA-repressed Gene(s) Involved in Recombination-dependent Mutation *via* Overexpression Although overproduction of RecA is insufficient to restore full levels of mutation of a *lexA3* strain (FIGURE II-2-A), the absolute dependence of adaptive mutagenesis on RecA (CHAPTER 2; Harris *et al.*, 1994; Foster, 1993, data not shown) suggests that de-repressed levels of this protein may, in fact, be necessary. This is supported by the observation that overproduction of RecA in an otherwise *rec*$^+$ *lex*$^+$ strain increases adaptive mutation (Cairns and Foster, 1991; FIGURE II-2-A), which suggests that RecA is limiting during adaptive mutation. Thus, transformation of a *lexA3 recAo281* strain with plasmids encoding known SOS genes and screening for mutation-restoration might reveal the LexA-repressed gene(s) which is necessary for full levels of adaptive mutation.

Thus far, attempts to restore mutability *via* overproduction have been unsuccessful. This can be attributed in part to the following quirks which should be avoided in future studies. First, at their recommended concentrations (Miller, 1992) many antibiotics (*e.g.* tetracycline, kanamycin), which are used commonly to maintain plasmids, kill *E. coli* during the conditions of adaptive mutation. Careful determinations of antibiotic concentrations suitable for the conditions of adaptive mutation should solve this problem. For example, one tenth the normal kanamycin concentration (5μg/ml) was used in experiments which assayed the effects of mismatch repair protein overproduction (CHAPTER 6). Second, many overexpression plasmids contain the *lacI* gene. This stimulates vast amounts of reversion to Lac$^+$ (most probably *via* recombination; data not shown), which prevent one from assaying adaptive mutation. Thus, *lac*-based expression systems should be avoided. Third, overexpressing some proteins can decrease a strain's viability significantly. For example, overproduction of RuvAB, even from a low copy plasmid, caused problems (Sharples *et al.*, 1990; Foster *et al.*, 1996). To avoid this problem, one could use constitutively-expressed chromosomal loci or plasmids with a low copy-number.

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[5] In other assay systems for adaptive mutation, defects in excision repair have increased (Cairns *et al.*, 1988; Hall, 1995; Prival and Cebula, 1992), decreased (F. Taddei, J.A. Halliday, I. Matic, M. Radman, personal communication), or not affected (Foster and Cairns, 1992) mutation.

Is *recF* the SOS Gene Required? A number of processes require function(s) encoded by *recF* (reviewed by Clark and Sandler, 1994). *recF* is essential for conjugational recombination in a *recB recC sbcB sbcC* strain[6]. *rec*$^+$ *E. coli* also require *recF* to some degree for plasmid (Kolodner *et al.*, 1985) and RecBCD-mediated (Miesel and Roth, 1996) recombination. In addition, *recF* may encode a function that facilitates DNA replication by assisting in reassembly of stalled replication forks (J. Courcelle, C. Carswell-Crumpton, P. Hanawalt, personal communication). Also, RecF functions in SOS induction, possibly by helping RecA become activated (see Hedge *et al.*, 1996, and references therein). All three of these processes require the RecA protein (Clark and Sandler, 1994). That RecF associates directly with RecA is supported by the isolation of mutations in *recA* that suppress some *recF* phenotypes (Clark, 1982; Volkert and Hartke, 1984; Madiraju *et al.*, 1988; Roca and Cox, 1990). Notably, *recA801* and *recA803* fail to suppress the SOS induction phenotype of *recF* mutants (Griffin and Kolodner, 1990).

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[6] *recB* (+/- *recC*) mutants are recombination deficient. Recombination proficiency can be restored fully by mutations in *sbcB* and *sbcC*, which are suppressors of *recBC*. However, mutations in *recF* abolish the recombination proficiency of the quadruple mutant, thus defining an alternative to the RecBCD recombination pathway which is commonly called the RecF recombination pathway (reviewed by Clark and Sandler, 1994).

Data in FIGURE II-3 and TABLE II-1 show that a *recF* null mutant displays about 2-fold less adaptive reversion than its *rec*$^+$ parent. This result is compatible with the hypothesis that *recF* is the LexA-repressed gene required in adaptive mutation, because the *recF* null mutant, like a *lexA3* mutant, is modestly hypomutable. This hypothesis awaits testing.

Speculation on a Function for RecF in Lac$^+$ Adaptive Mutation Recombination, DNA synthesis, and SOS induction (above) are required for efficient adaptive mutagenesis (summarized in CHAPTER 7) and all are affected by *recF*. This makes it difficult to pinpoint the role(s) of *recF* in adaptive mutation. Recall that RecBC(D) is required for Lac$^+$ adaptive mutation (CHAPTER 2, Harris *et al.*, 1994). RecD inhibits the recombination activity of RecBC such that a *recD* mutant is hyper-recombinagenic (Amundsen *et al.*, 1986; Biek and Cohen, 1986; Thaler *et al.*, 1989) and hypermutable adaptively (CHAPTER 2, Harris *et al.*, 1994). SOS induction produces a *recD* mutant phenocopy (Kannan and Dharmalingam, 1990; Rinken and Wackernagel, 1992). We hypothesized that SOS-dependent RecD-inactivation is required for full levels of adaptive mutation. If so, then mutating a gene required for SOS-inactivation of *recD* should diminish adaptive mutation. I hypothesized that RecD-inactivation during adaptive mutation requires RecF. This predicts that a *recD recF* double mutant should be as hypermutable adaptively as a *recD* single mutant. Data in FIGURE II-4 confirm this prediction. This result suggests that RecF facilitates RecD inactivation in this assay system. Although this result is consistent with the notion that *recF* is the LexA-repressed

SOS gene, such a conclusion can not be made until, for example, overproduction of RecF or inactivation of *recD* is shown to restore the mutability of a *lexA3 recAo281* strain.

To attempt to generalize the apparent role of RecF in RecD inactivation, I hypothesized that RecF is also necessary for RecD inactivation at Chi (χ) sequences. A current model for RecBC(D)-mediated recombination proposes that RecBCD degrades double-strand DNA until it reaches χ, whereupon the enzyme becomes a DNA helicase required for recombination (Rosenberg and Hastings, 1991). The molecular basis for this switch in activities at χ is thought to be RecD inactivation (Thaler *et al.*, 1989; Rosenberg and Hastings, 1991; Rinken and Wackernagel, 1992; Dixon and Kowalczykowski, 1993; Koppen *et al.*, 1995; Myers *et al.*, 1995), but the exact mechanism of this inactivation is unknown. If RecF is required for this interaction, then a *recF* mutant should lack χ-stimulated recombination.

To test this hypothesis, $\lambda(\chi^{+/o})$ by λ crosses (Razavy *et al.*, 1996) were performed in *rec*$^+$, *recF*, *recD*, and *recF recD E. coli* strains. *recF* and *rec*$^+$ strains showed similar χ-dependent stimulations of homologous recombination (FIGURE II-5), which would not have occurred had RecF been necessary for RecD inactivation at χ. Also, χ had no effect in a *recD* or *recF recD* strain indicating that this assay is sensitive to RecD inactivation (FIGURE II-5; also see Razavy *et al.*, 1996). Thus, *recF* is not necessary for RecD inactivation at χ.

FURTHER DISCUSSION

An Alternative Interpretation of the Effect of *lexA3* on Adaptive Lac Reversion A role for SOS induction in adaptive mutation is one interpretation of the *lexA3* result which implies that de-repression of the LexA regulon is required for adaptive mutation (FIGURE II-2). Alternatively, *lexA3* may encode a super-repressor that diminishes the expression of LexA-regulated proteins to levels lower than those present in uninduced cells (*i.e.* less than basal levels; R. Woodgate, personal communication). Thus, any component of the LexA regulon whose basal levels of expression are required for adaptive mutation could be limiting in a *lexA3* strain (*e.g. ruvAB* or *recF*).

This alternative hypothesis could be addressed *via* overproduction of candidate SOS proteins in a *recA430* mutant which is recombination-proficient (Morand *et al.*, 1977; Roca and Cox, 1990), unable to facilitate the cleavage of LexA (Walker, 1996), and hypomutable adaptively (Cairns and Foster, 1991; Harris *et al.*, 1994), presumably because of its inability to process LexA (but see below). If the overproduced SOS protein restored the mutability of a *lexA3 recAo281* strain and of a *recA430* strain, then it could be concluded that the SOS response has a *bona fide* role in Lac$^+$ adaptive mutation. However, if only the mutability of the *lexA3 recAo281* strain was restored, then the effect of *lexA3* could be due simply to reduced basal expression of the LexA-regulon. A potential drawback to this approach is the possibility that the RecA430 protein may be defective in co-cleavage of another, as yet, unidentified protein which could also be required in its cleaved form for full levels of adaptive mutation. In support of this, the depression of adaptive mutation caused by *recA430* is greater than that caused by *lexA3* (Cairns and Foster, 1991). This disparity is probably not due to decreased recombination ability, as *recA430* is recombination-proficient (Morand *et al.*, 1977; Roca and Cox, 1990).

Observations on Constitutive De-repression of the LexA Regulon If the role of SOS induction in adaptive mutagenesis is simply for overexpression of one or more genes of the LexA regulon, then constitutive de-repression of the LexA regulon should elevate mutation. LexA-deficient, LexA(def), strains require inactive *sulA* for viability. *sulA* encodes a protein that blocks cell division in response to DNA damage (Walker, 1996). Inactivation of *sulA* did not significantly affect Lac+ adaptive mutation (TABLE II-2). Surprisingly, a *lexA*(def) mutation (*lexA71*::Tn*5*, Kruger *et al.*, 1983) caused a 2-fold drop in Lac+ adaptive mutation of a *sulA* strain (TABLE II-2). Constitutive de-repression of the SOS regulon appears to inhibit Lac+ adaptive mutation. This could be for a number of reasons. Perhaps a precise level of the required LexA-repressed gene product is necessary. Alternatively, the transposon insertion in *lexA* also inactivates a downstream gene, *dinF*, the function of which is not yet known. Further work is required to assess whether a loss of dinF gene product depresses Lac+ adaptive mutation.

CONCLUSIONS

The SOS response is likely to play a role in Lac+ adaptive mutation. *recF*, a LexA-regulated gene, is required for efficient Lac+ adaptive mutation. RecF exerts its effect upstream of RecD in an epistasis pathway. Thus, *recF* could be one of several candidate genes whose SOS-induced expression is required. Further work is needed to identify the genes involved.

TABLE II-1. Effect of *recF* on Lac$^+$ adaptive mutation.

Strain[a]	Expt.	Cumulative number of Lac$^+$ colonies by day 5 per 10^8 viable cells[b] (mean ± SEM)[c]	Decrease in Lac$^+$ adaptive mutation relative to *rec*$^+$ strain: Within each expt.	Decrease in Lac$^+$ adaptive mutation relative to *rec*$^+$ strain: Average (mean ± SEM)
rec$^+$	1	75 ± 5.4	1	1
	2	140 ± 7.5	1	
	3	25 ± 1.5	1	
	4	5.9 ± 0.7	1	
	5	21 ± 4.9	1	
	6	23 ± 3.6	1	
	7	56 ± 5.5	1	
	8	49 ± 5.3	1	
recF	1	25 ± 0.6	0.33	0.48 ± 0.05
	2	50 ± 3.8	0.36	
	3	12 ± 0.9	0.48	
	4	3.8 ± 0.7	0.64	
	5	8.2 ± 0.8	0.39	
	6	16 ± 1.0	0.70	
	7	23 ± 2.0	0.41	
	8	27 ± 1.9	0.55	

[a] *rec*$^+$ and *recF* are SMR506 and SMR686 (APPENDIX I).

[b] In each experiment the mean number of Lac$^+$ colonies was determined from 4-12 independent cultures of each strain. Jackpots of growth-dependent mutants, as defined in CHAPTER 1, were excluded from the calculations.

[c] SEM, one standard error of the mean.

TABLE II-2. Effects of *sulA211* and *lexA71* on Lac$^+$ adaptive mutation.

Strain[a]	Expt.	Cumulative number of Lac$^+$ colonies by day 5 per 10^8 viable cells[b] (mean ± SEM)[c]	Decrease in Lac$^+$ adaptive mutation relative to control strain[d]: Within each expt.	Decrease in Lac$^+$ adaptive mutation relative to control strain[d]: Average (mean ± SEM)
sul$^+$ *lex*$^+$	8	49 ± 5.3	1	1
	9	40 ± 9.4	1	
	10	31 ± 5.9	1	
	11	60 ± 12	1	
sulA211 lex$^+$	8	27 ± 5.3	0.55	1.2 ± 0.28
	9	74 ± 9.0	1.9	
	10	37 ± 3.8	1.2	
	11	78 ± 11	1.3	
sulA211 lexA71	9	24 ± 2.5	0.32	0.45 ± 0.15
	10	28 ± 5.0	0.76	
	11	22 ± 3.8	0.28	

[a] *sulA*$^+$ *lexA*$^+$, *sulA211 lexA*$^+$, and *sulA211 lexA71* are SMR506, SMR1827, and SMR2601 (APPENDIX I).

[b] In each experiment the mean number of Lac$^+$ colonies was determined from 10 independent cultures of each strain. Jackpots of growth-dependent mutants, as defined in CHAPTER 1, were excluded from the calculations.

[c] SEM, one standard error of the mean.

[d] The *sulA211 lexA*$^+$ strain is compared with the *sulA*$^+$ *lexA*$^+$ strain, and the *sulA211 lexA71* strain is compared with the *sulA211 lexA*$^+$ strain.

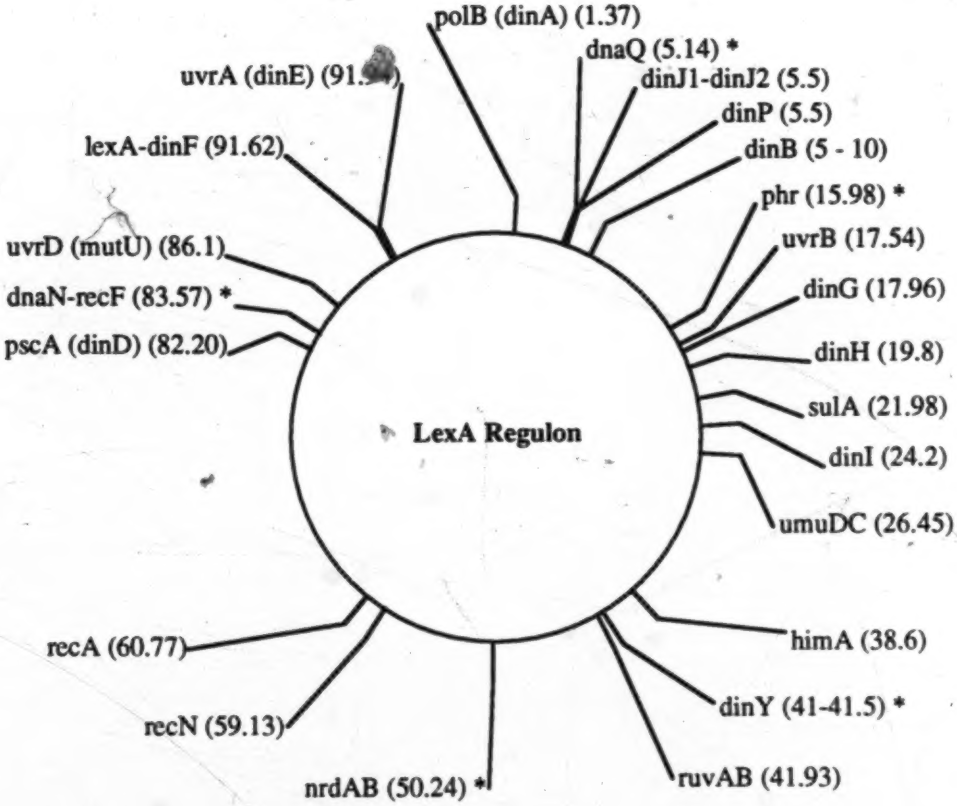

polB (dinA) (1.37)
dnaQ (5.14) *
dinJ1-dinJ2 (5.5)
dinP (5.5)
dinB (5 - 10)
phr (15.98) *
uvrB (17.54)
dinG (17.96)
dinH (19.8)
sulA (21.98)
dinI (24.2)
umuDC (26.45)
himA (38.6)
dinY (41-41.5) *
ruvAB (41.93)
nrdAB (50.24) *
recN (59.13)
recA (60.77)
pscA (dinD) (82.20)
dnaN-recF (83.57) *
uvrD (mutU) (86.1)
lexA-dinF (91.62)
uvrA (dinE) (91.[illegible])
LexA Regulon

FIGURE II-1. The LexA regulon. DNA damage-inducible (*din*) genes (Fogliano and Schendel, 1981; Kannan and Dharmalingam, 1990; Kenyon and Walker, 1980; Kenyon and Walker, 1981; Lewis *et al.*, 1994; Lewis *et al.*, 1992; Miller *et al.*, 1981; Ohmori *et al.*, 1995; Pax-Elizur *et al.*, 1996; Sancar *et al.*, 1982; Siegel, 1983; Walker, 1996; Witkin, 1991 and references therein) and their approximate chromosomal positions in minutes (Berlyn, 1996; Berlyn *et al.*, 1996). Although DNA damage-inducible, not all SOS genes have *din* designations. Unless marked otherwise, genes are repressed directly by LexA and induced by RecA-dependent cleavage of this repressor. Genes marked by a single asterisk (*dnaQ*, *phr*, *dinY*, *nrdAB*) are regulated by RecA and LexA, but are probably not repressed directly (Walker, 1996).

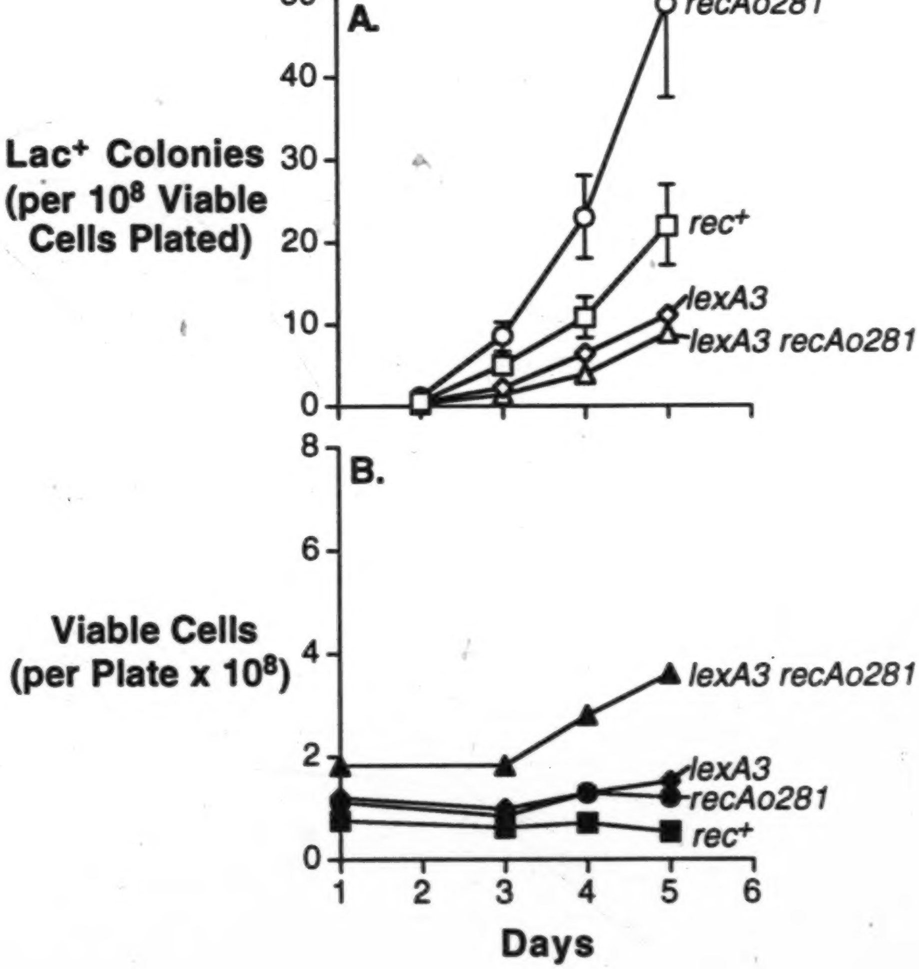


FIGURE II-2. Overproduction of RecA does not to restore adaptive mutability of a *lexA3* strain. **A.** Overproduction of RecA fails to increase the mutability of a *lexA3* strain. Error bars represent one SEM (not visible if smaller than the data point). **B.** For all strains the number of viable cells on each plate varied less than 2-fold over the duration of the experiment. The *rec*+, *lexA3*, *recAo281*, and *lexA3 recAo281* strains correspond to SMR506, SMR841, SMR846, and SMR845 respectively (APPENDIX I). The presence of each of these mutations was confirmed by its characteristic UV phenotype (see APPENDIX I).

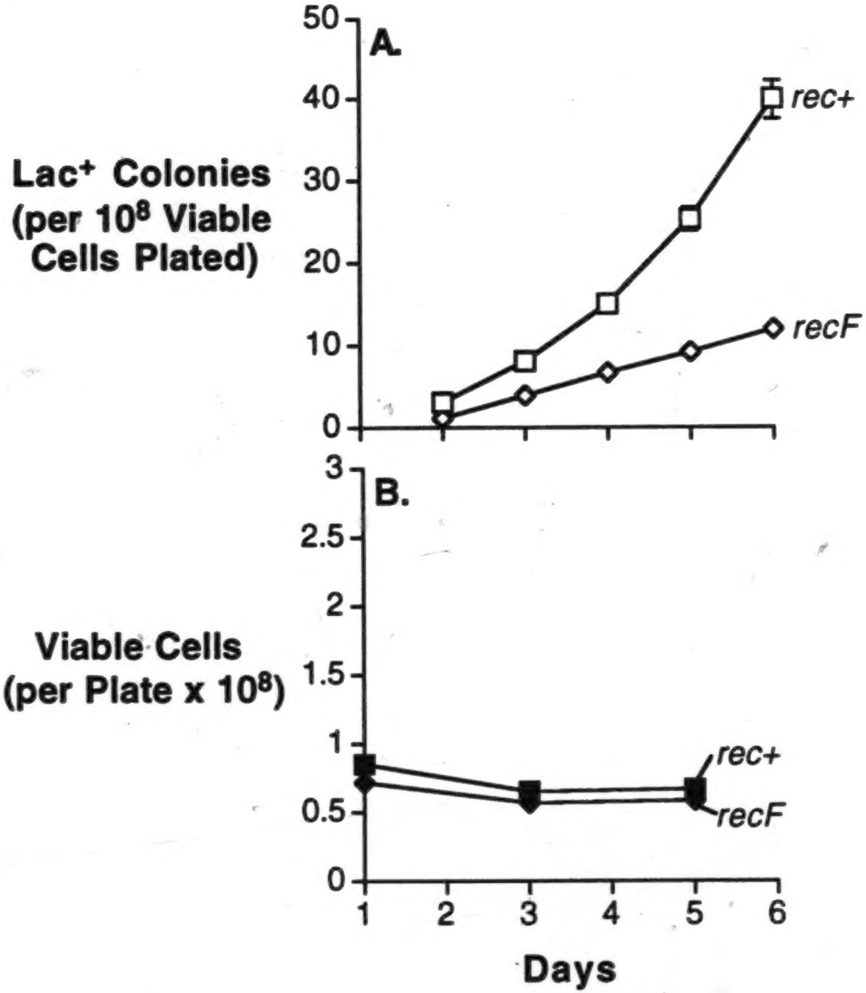


FIGURE II-3. RecF is required for efficient Lac$^+$ adaptive mutation. A. A *recF* null mutant displays 2-3-fold less reversion to Lac$^+$ than its *rec*$^+$ parent. These data are an alternate representation of those in TABLE II-1 experiment 3. Error bars represent one SEM (not visible if smaller than the data point). **B.** The number of viable *rec*$^+$ or *recF* frameshift-bearing cells varied less than 2-fold. Thus, a change in the number of cells on the plates can not account for the observed difference in mutability. Strains *rec*$^+$ and *recF* are SMR506 and SMR686 respectively (APPENDIX I). See APPENDIX I for confirmation of the genotype of SMR686.

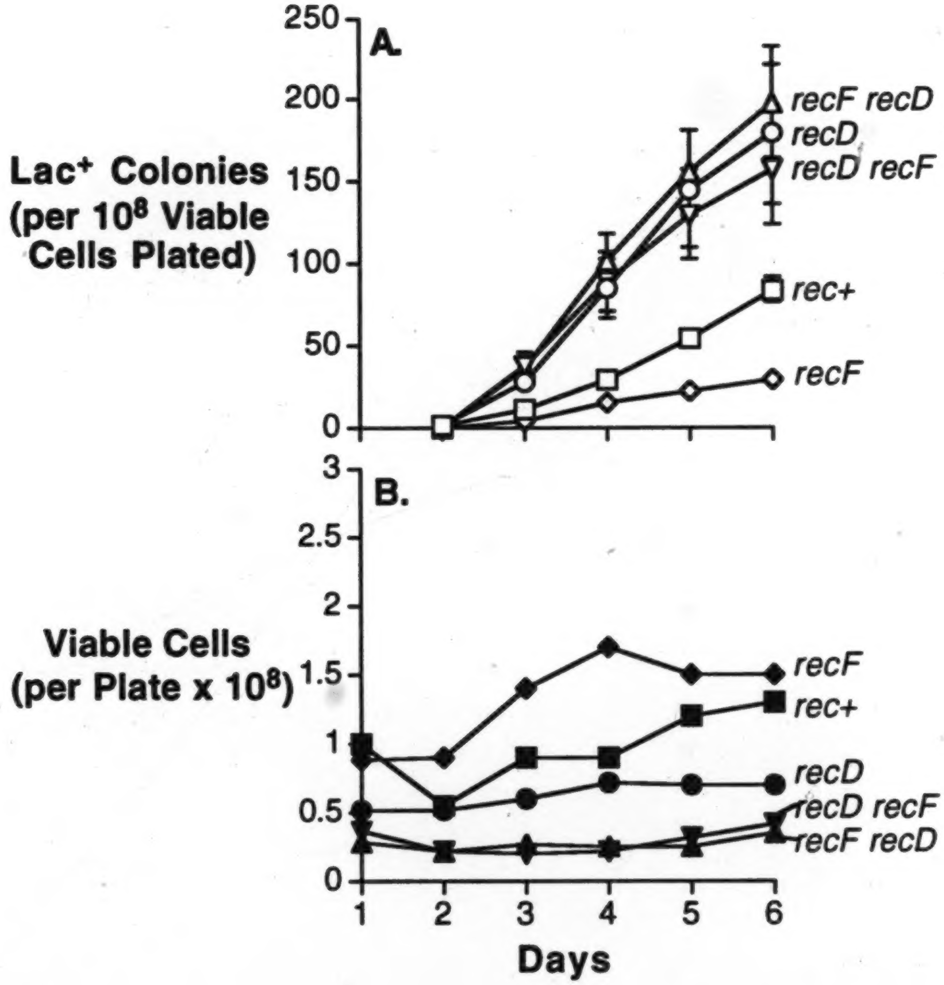
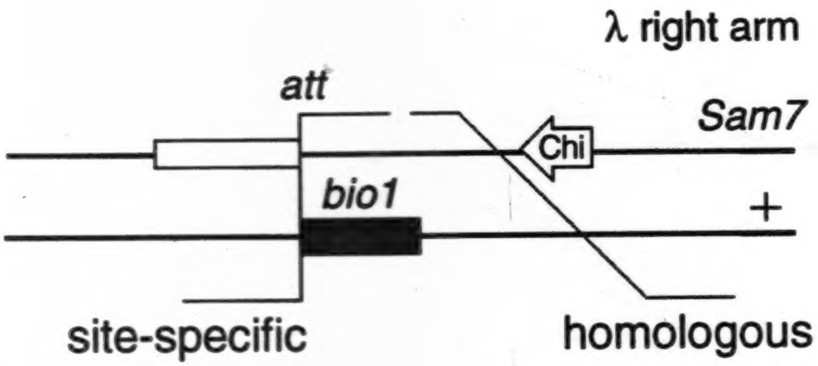
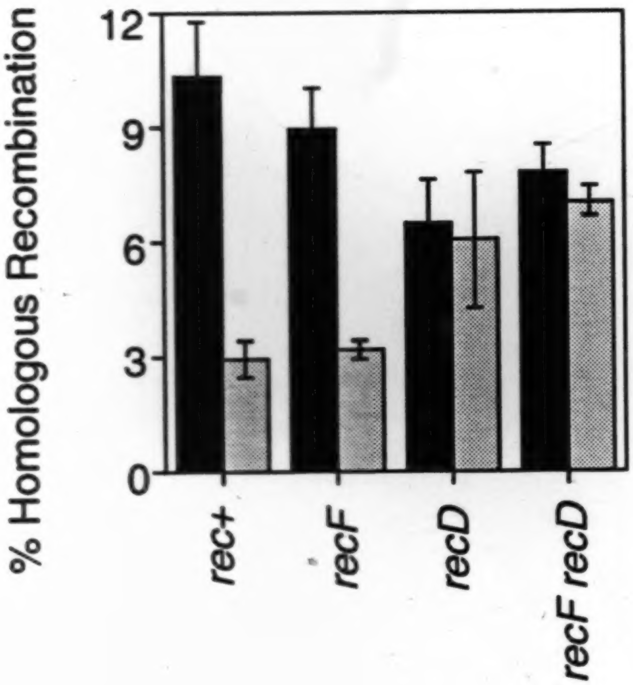


FIGURE II-4. A. *recD* is epistatic to *recF*. Error bars represent one SEM (not visible if smaller than the data point). **B.** For all strains, the number of viable cells on the plates varied less than 2-fold through the experiment's duration. Strains *rec*$^+$, *recF*, *recD*, *recD recF* and *recF recD* are SMR506, SMR686, SMR692, SMR1496, and SMR1497 respectively (APPENDIX I). The *recD recF* and *recF recD* strains are genotypically identical but were constructed differently (APPENDIX I). See APPENDIX I for genotypic confirmation of SMR686, SMR692, SMR1496, and SMR1497.

FIGURE II-5. Percent recombination in *recF*- and/or *recD*-defective cells. Crosses measuring recombination in the λ right arm were performed in *rec*$^+$, *recF*, *recD*, and *recF recD* strains (SMR506, SMR686, SMR692, and SMR1497, respectively; APPENDIX I) as described by Razavy *et al.* (1996). Phage genotypes are λ *Δb527 red3 gam210 cIts857 ChiC* (Chi$^+$ parent only) *Δnin5 Sam7* [top λs: λ SR324 (Chi$^+$) and λ SR325 (Chi°), lab collection) and λ *bio1 Δnin5* (bottom λ: λ SR27, lab collection). The deletion-free site-specific recombinants in the densest peak of the density gradient were plated on SuIII$^+$ *recA* (KR3A, lab collection) for total phage and on SuII$^+$ *recA* (C600 *recA*, lab collection) for S^+ homologous recombinants. Each bar represents the mean percent (± one standard deviation) of S^+ homologous recombinants in at least 2 fractions from the densest peak of one cross and density gradient. All crosses were performed in parallel. Dark and light bars represent data from Chi$^+$ and Chi° crosses, respectively, and a difference between these values indicates Chi-stimulation of recombination.


λ right arm
att
Sam7
Chi
bio1
+
site-specific
homologous


% Homologous Recombination
12
9
6
3
0
rec+
recF
recD
recF recD

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APPENDIX III

ADDITIONAL STUDIES ON DNA POLYMERASES INVOLVED IN RECOMBINATION-DEPENDENT MUTATION

INTRODUCTION

Recombination-dependent adaptive reversions of the *lacI33ΩlacZ* frameshift mutation are mostly -1 deletions in mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg *et al.*, 1994). This spectrum suggests DNA polymerase errors (Ripley, 1990) which probably occur *via* a template-slippage mechanism (Streisinger *et al.*, 1966). If so, then which of *E. coli*'s three DNA polymerases is responsible?

To address this question, polymerase mutants were constructed and examined for their ability to revert to Lac$^+$. *E. coli* DNA polymerase I, II, and III (PolI, PolII, and PolIII) are encoded by the *polA*, *polB*, and *dnaE* (*polC*) genes, respectively (Kornberg and Baker, 1992, pp.159-172 and references therein). Mutations in *polA* or *polB* stimulate Lac$^+$ adaptive reversion, whereas a mutation in *dnaE* decreases Lac$^+$ adaptive reversion (data and references below and in CHAPTER 4). These results imply that all three DNA polymerases perform DNA synthesis during adaptive mutation and support the idea that PolIII generates the errors that lead to recombination-dependent adaptive mutations.

RESULTS AND DISCUSSION

DNA Polymerase I in Lac$^+$ Adaptive Mutation[1] PolI functions in gap filling in DNA replication and DNA repair (Kornberg and Baker, 1992). *polA* mutants show increased sensitivity to DNA damaging agents (*e.g.* UV light), a sensitivity to rich media, increased recombination, plasmid instability, deficiencies in phage replication and recombination, and inviability as *lig*, *recA*, *recB*, and *uvrB* double mutants (*polA* mutant phenotypes and references listed in Table 4-9 of Kornberg and Baker, 1992). The latter phenotype suggests that functions of PolI and those of DNA ligase I, RecA, RecB, and UvrB are in some way redundant (Cao and Kogoma, 1995).

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[1] The data in TABLE III-1 and FIGURE III-1 were obtained by Roger Sidhu, an undergraduate research student, under the supervision of R.S. Harris.

Several attempts at P1 transducing a *ΔpolA*::*kan* null allele from CJ300 (Joyce and Grindley, 1984, SMR785, APPENDIX I) into FC40 (SMR506, APPENDIX I) yielded no Kan^r transductants (see SMR506, TABLE III-1). However, P1 transduction of this null mutation, with the same P1 lysates, into other genetic backgrounds produced hundreds of Kan^r transductants which showed *polA* phenotypes [data not shown; *e.g.* transduction into C600 (SMR127, APPENDIX I) and W3110 (SMR455, APPENDIX I) produced SMR893 and SMR892, respectively, APPENDIX I]. These observations imply that *polA*::*kan* renders FC40 inviable.

Data in TABLE III-1 indicate that strains carrying the F' episome of FC40 are inviable in combination with a *polA* deletion. Absence of *polA* could result in loss of the F' plasmid, which causes post-segregational killing of the host cell (Jensen and Gerdes, 1995).

To avoid the problem of the apparent inviability of FC40 *ΔpolA*, a conditional *polA* allele, *polA12*(Ts) (Kornberg and Baker, 1992) was used. *polA12* confers a hypersensitivity to UV and methyl methanesulfonate at 43°C. The mutant polymerase encoded by *polA12* lacks nick translation activity at 43°C and is somewhat deficient even at 30°C (Kornberg and Baker, 1992). To avoid the potentially lethal combination of a *polA* mutation and a F' plasmid, all strains were constructed at 30°C and adaptive mutation experiments performed at 37°C. Data in FIGURE III-1 show that *polA12* increases RecA-dependent Lac$^+$ adaptive mutation 2-fold. The transposon in the *fadAB* operon that was used to mobilize the *polA* mutation (*fadAB3165*::Tn*10kan*) has little affect on Lac$^+$ adaptive mutation (FIGURE III-1). These data suggest that *polA12* increases *recA*-dependent adaptive mutation. However, it is possible that *polA12* also increases rates of growth-dependent *lac* reversion, in which case the effect would not be specific to adaptive mutation. This is probably not the case as all strains displayed similar levels of reversion at early time points of the experiment suggesting that *polA12* is not an obvious mutator during growth. Nevertheless, experiments to measure rates of growth-dependent *lac* reversion must be done.

For now, the *polA12* data imply, first, that PolI is not the DNA polymerase that makes errors that lead to *rec*-dependent adaptive mutations, because a decrease in reversion to Lac$^+$ is not observed, and second, that PolI may compete with the DNA polymerase that makes Lac$^+$ adaptive mutations. Alternatively, *polA12* may increase mutation by stimulating recombination or another RecA-dependent route of mutation.

DNA Polymerase II in Lac$^+$ Adaptive Mutation PolII is encoded by the LexA-repressed *polB* gene (Bonner *et al.*, 1990; Iwasaki *et al.*, 1990). PolII facilitates the bypass of abasic lesions *in vivo* in SOS-induced cells (Tessman and Kennedy, 1994). A *polB* proofreading-defective mutant displays increased rates of mutation in chromosomal and F'-located genes (Rangarajan *et al.*, 1997) suggesting that PolII plays a role in chromosomal and episomal DNA synthesis *in vivo* in SOS-uninduced cells. When this study was undertaken, it was possible that *polB* was the LexA-repressed gene, whose induced expression facilitates adaptive mutation. This turned out not to be the case as a *polB* null mutation stimulates Lac$^+$ adaptive mutation (Escarcellar *et al.*, 1994, and below).

The *polBΔ1*::Ω Sm-Sp null allele (*polBΔ1*, Escarcellar *et al.*, 1994) which confers resistance to spectinomycin (Spc^r), was transduced into the *lac* frameshift-bearing strain and Spc^r transductants were assayed for Lac$^+$ adaptive mutation. Others have reported that PolII mutants show no obvious growth or replication defects (Kornberg and Baker, 1992; Kornberg and Baker, 1992). However, upon purification on rich medium containing spectinomycin, Spc^r transductants produced small- and large-sized colonies. This suggested that *polBΔ1* confers a growth defect (at least in our strain) and that the large colonies represent cells that harbor growth-defect-suppressing mutations. One small and one large colony-forming *polBΔ1* isolate, which were derived from a single Str^r transductant, were assayed for Lac^+ adaptive reversion (FIGURE III-2).

The small colony-forming *polBΔ1* isolate shows an approximately 3-fold increase in Lac^+ adaptive mutation (FIGURE III-2, A and B), whereas the large colony-forming *polBΔ1* isolate displays a 3- to 5-fold decrease in Lac^+ adaptive mutation (FIGURE III-2, A and B). No obvious correlation exists between the number of viable frameshift-bearing cells throughout the experiment's duration (FIGURE III-2-C) and Lac^+ adaptive mutation, indicating that the differences in mutability can not be attributed to growth or death. Growth-dependent reversion to Lac^+ appears unaffected by *polBΔ1* as the numbers of early-arising Lac^+ colonies are unchanged by this mutation (see also Foster *et al.*, 1995). The results suggest that the *polB* null phenotype is elevated Lac reversion; however, conclusions based on these data could not be made without determining whether the Str^r isolates are *bona fide polB* mutants and/or whether they contain growth-defect-suppressing mutations.

To confirm the presence of *polBΔ1* and to attempt to separate this mutation from potential second-site suppressors, *polBΔ1* was backcrossed into FC40 from both the small and large-colony forming isolates. Two independent, small colony Spc^r transductants derived from each isolate (*i.e.* 4 separate transductants) displayed Lac^+ adaptive hypermutation similar to the original small colony-forming isolate (*e.g.* FIGURE III-3-A). Again, frequencies of early-arising Lac^+ colonies appeared similar and little change in the numbers of frameshift-bearing cells occurred through the experiment's duration (FIGURE III-3-B). Thus, both the small and large-colony forming isolates are *bona fide polBΔ1* mutants, but the large-colony forming isolate probably contains a growth-defect-suppressing mutation as the hypermutable (not the hypomutable) phenotype transduced with the *polBΔ1* mutation. This strain has not been characterized further.

In summary, the 3-fold increase in Lac$^+$ adaptive mutation caused by the absence of PolII (FIGURES III-2 and III-3; see also Escarcellar *et al.*, 1994; Foster *et al.*, 1995) suggests, first, that PolII is not the DNA polymerase that makes the errors that become Lac$^+$ adaptive mutations, and second, that PolII, like PolI, appears to compete with the DNA polymerase that makes Lac$^+$ adaptive mutation. Such a polymerase-competition hypothesis predicts that the elevation of adaptive mutation in the *polBΔ1* strain is *recA*-dependent (*i.e.* that *polBΔ1* is not activating a novel pathway of mutation-formation). This remains to be tested. Finally, if one assumes that *E. coli* has only three DNA polymerases then these data and those of PolI above support the interpretation discussed in CHAPTER 4 that DNA PolIII makes Lac$^+$ adaptive mutations.

DNA Polymerase III in Lac$^+$ Adaptive Mutation PolIII holoenzyme consists of at least 10 separate subunits and is the major replicative DNA polymerase of *E. coli* (Kornberg and Baker, 1992). The catalytic core of DNA polymerase III is composed of 3 subunits: (i) the α or polymerase subunit, encoded by *dnaE*; (ii) the ε or proofreading exonuclease subunit, encoded by *dnaQ* (*mutD*); and (iii) the θ subunit (function unknown), encoded by *holE* (Slater *et al.*, 1994). The most convincing evidence for a direct role of PolIII in Lac$^+$ adaptive mutation is presented in CHAPTER 4. Additional work with a PolIII-specific component follows.

Some of the subunits of the PolIII holoenzyme are not required for DNA synthesis or cell viability (*e.g.* θ, Slater *et al.*, 1994). However, their absence may affect Lac$^+$ adaptive mutation. An effect would implicate PolIII in mutation-formation. No effect would show only that a subunit is not essential for adaptive Lac reversion. Data in FIGURE III-4 show that the absence of the θ subunit of the PolIII holoenzyme has little effect on recombination-dependent adaptive mutation. Therefore, θ is not necessary for Lac$^+$ adaptive mutation.

SUMMARY AND FURTHER DISCUSSION

The data presented here and those in CHAPTER 4 imply strongly that DNA polymerase III generates errors that become recombination-dependent adaptive mutations. A loss of function mutation in *polA*, *polA12*, and a null mutation in *polB*, *polBΔ1*, elevate levels of Lac$^+$ adaptive mutation (this appendix), whereas an antimutator allele of *dnaE*, *dnaE915*, diminishes Lac$^+$ adaptive mutation even in the absence of mismatch repair (CHAPTER 4). These results suggest that all three DNA polymerases perform DNA synthesis under conditions of adaptive mutation and, perhaps, compete with each other during adaptive mutation. DNA synthesis by PolIII, however, may be more error-prone, as absence of either PolI or PolII increases in Lac$^+$ adaptive mutation.

This work also produced an unexpected, but provocative, result: a *polBΔ1* growth-defect-suppressing mutation appears to abolish Lac$^+$ adaptive mutation. In which gene does this mutation map -- to *dnaE*, to a *rec* gene required for Lac$^+$ adaptive mutation, or to an as yet unidentified gene? Would this mutation also abolish Lac$^+$ adaptive mutation in a *polB*$^+$ strain and thereby reveal another genetic requirement for adaptive mutation? Characterization of this suppressor could identify additional players in the molecular mechanism of recombination-dependent mutation and/or components between recombination and DNA synthesis.

TABLE III-1. Inability to construct F'-bearing *polA* strains[a].

Strain[b]	Genetic background[c]	Relevant genotype	No. Kan^r transductants scored	
			Expt. 1	Expt. 2
SMR829	C600	*Δ(proAB-lac)*XIII	53	130
SMR830	C600	*proAB*$^+$ *lacY1*	64	174
SMR1305	C600	*Δ(proAB-lac)*XIII [F' *proAB*$^+$ *lacI33ΩlacZ*]	0	1
SMR505	P90C	*Δ(proAB-lac)*XIII	18	154
SMR506	P90C	*Δ(proAB-lac)*XIII [F' *proAB*$^+$ *lacI33ΩlacZ*]	0[d]	0[d]

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[a] Standard bacterial protocols were employed (Miller, 1992). 0.5 ml of P1 grown on CJ300 (*ΔpolA*::*kan*, Joyce and Grindley, 1984) was used to transduce each of the strains below. Separate lysates were used for experiments 1 and 2. One hour was allowed for expression of Kan^r prior to plating on minimal M9 plates supplemented with 0.2% glucose, 10μg/ml thiamine, 50μg/ml threonine, 50μg/ml leucine, 50μg/ml proline, 0.6% sodium citrate, and 50μg/ml kanamycin. Kan^r transductants were scored after 48 hours of incubation at 37°C. Viabilities of the recipient strains were similar.

[b] See APPENDIX I for full genotypes, construction details, and references.

[c] C600 is SMR127 and P90C is SMR503 (APPENDIX I).

[d] These data were obtained in reconstruction experiments using the same P1 lysates and similar conditions.

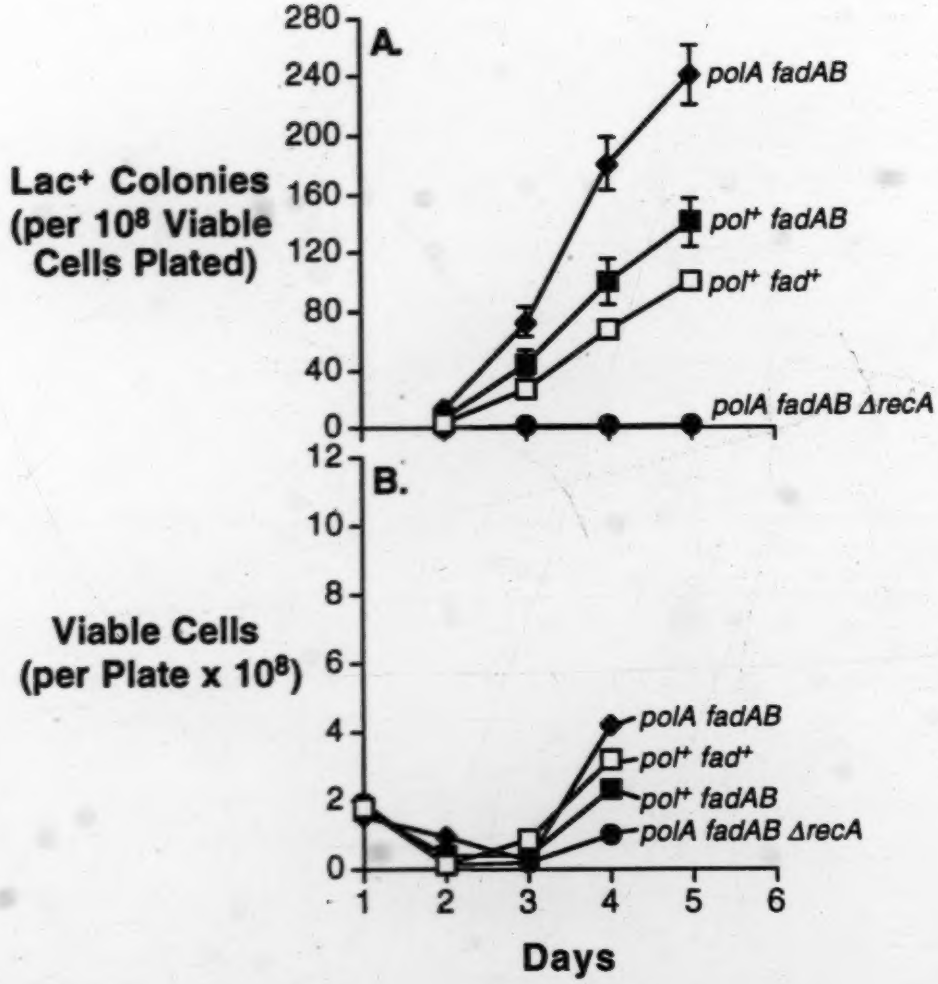
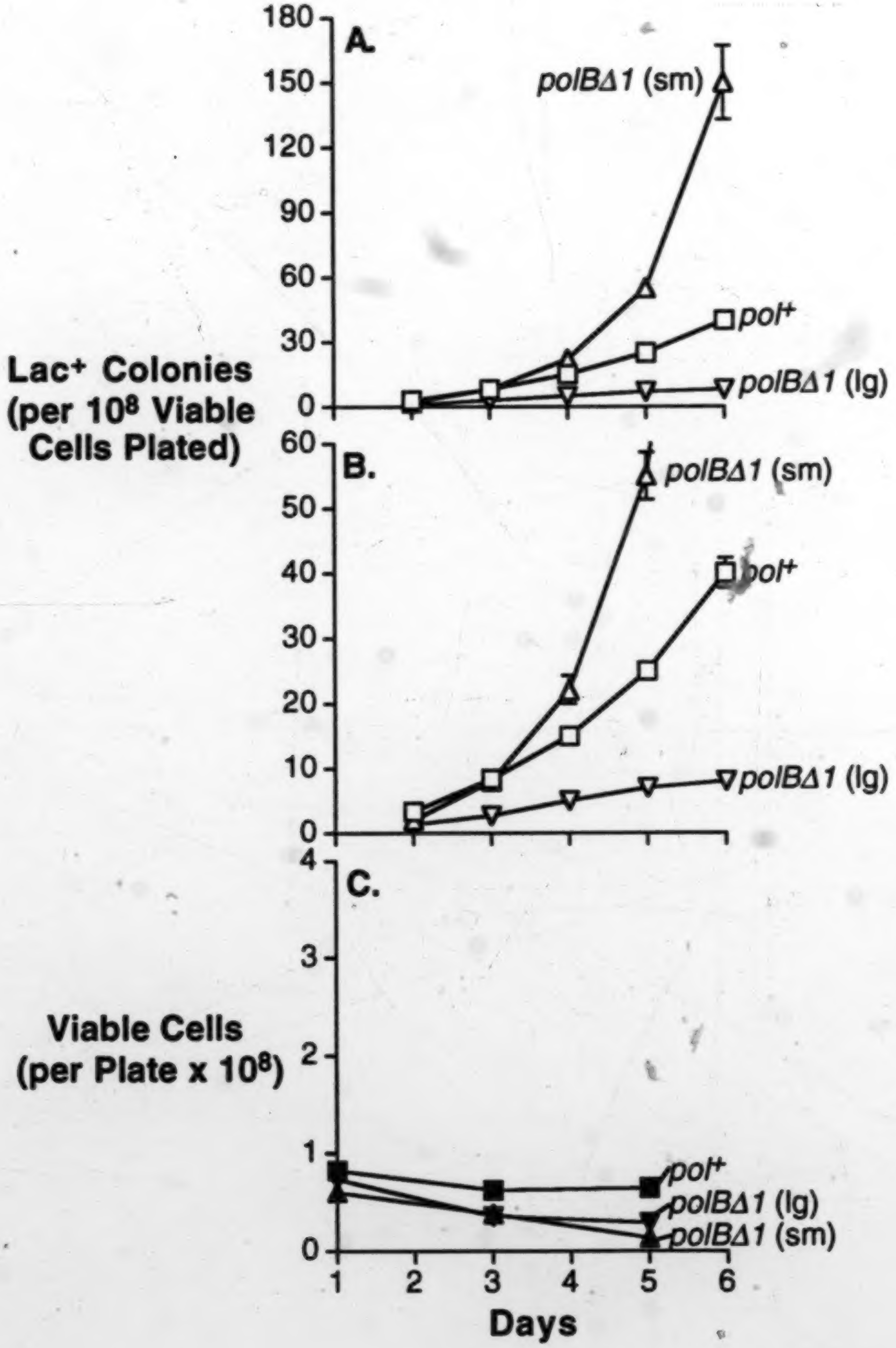


FIGURE III-1. A. A *polA*(Ts) mutant shows a 2-fold increase in *recA*-dependent Lac$^+$ adaptive mutation. Error bars represent one SEM (not visible if smaller than the data point). **B.** The number of viable frameshift-bearing cells declined until day 3 and recovered on day 4 for all of the strains. Thus, the apparent differences in adaptive *lac* reversion cannot be attributed easily to growth or death of the frameshift-bearing cells. The *polA fadAB*, *pol$^+$ fadAB*, *pol$^+$fad$^+$*, and *polA fadAB ΔrecA* strains correspond to SMR3491, SMR3490, SMR506, and SMR3492, respectively (APPENDIX I).

FIGURE III-2. Effects of a *polB* null mutation on Lac$^+$ adaptive mutation. **A.** A small colony-forming *polBΔ1* isolate displays a 3-fold increase in Lac$^+$ adaptive mutation, whereas a large colony-forming *polBΔ1* isolate, which may harbor a growth-defect-suppressing mutation (see text), shows a 3- to 5-fold decrease in Lac$^+$ adaptive mutation. **B.** The data are identical to those in A., except the Y-axis of the graph has been expanded to illustrate the difference between the *pol*$^+$ strain and the large colony-forming *ΔpolB1* strain. **C.** A slight decrease in the numbers of viable frameshift-bearing cells occurred during the experiment. This decrease is not sufficient to account for the large differences in adaptive Lac reversion as there is no apparent correlation between mutability and viability. Error bars represent one SEM (not visible if smaller than the data point). The *pol*$^+$, *polBΔ1*(sm), and *polBΔ1*(lg) strains correspond to SMR506, SMR3658, and SMR3659, respectively (APPENDIX I).


180
150
120
90
60
30
0
A.
polBΔ1 (sm)
pol+
polBΔ1 (lg)
Lac+ Colonies
(per 10^8 Viable
Cells Plated)
60
50
40
30
20
10
0
B.
polBΔ1 (sm)
pol+
polBΔ1 (lg)
4
3
2
1
0
C.
Viable Cells
(per Plate x 10^8)
pol+
polBΔ1 (lg)
polBΔ1 (sm)
1
2
3
4
5
6
Days

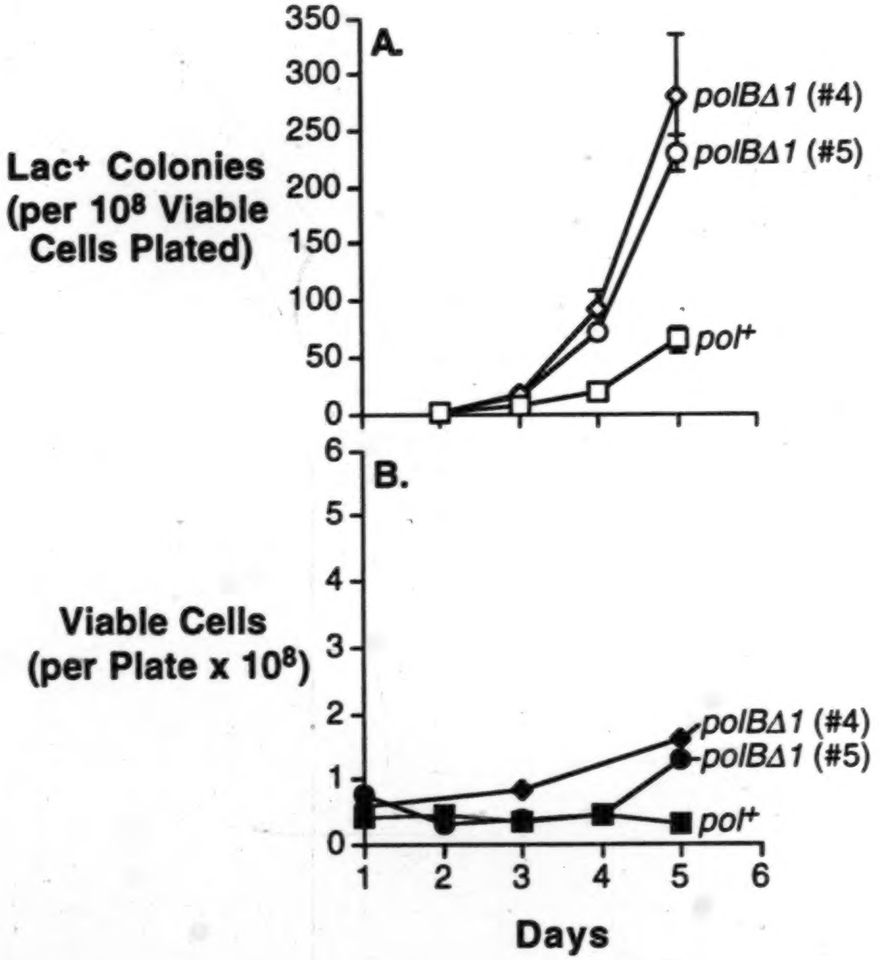


FIGURE III-3. ***polBΔ1*** **causes a 3-fold increase in Lac+ adaptive mutation.** **A.** Two independently constructed *polBΔ1* isolates show a 3-fold increase in Lac+ adaptive mutation. The small and large colony forming *polBΔ1* strains in FIGURE III-2 served as P1 donors for the construction of *polBΔ1* isolates #4 and #5, respectively (see APPENDIX I for construction details). **B.** A slight change in the numbers of viable frameshift-bearing cells occurred during the experiment. Error bars represent one SEM (not visible if smaller than the data point). The *pol+*, *polBΔ1*(#4), and *polBΔ1*(#5) strains correspond to SMR506, SMR3661, and SMR3662, respectively (APPENDIX I).

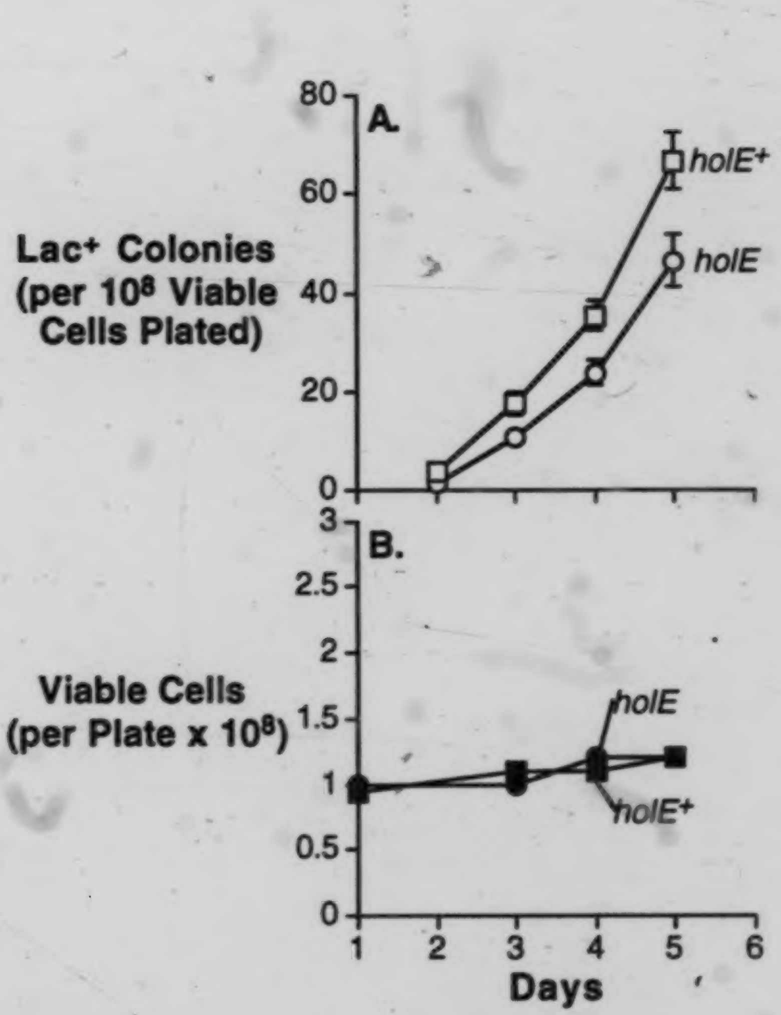


FIGURE III-4. Absence of the θ subunit of the PolIII holoenzyme does not affect Lac$^+$ adaptive mutation. A. A *holE* null mutant displays only a slight decrease in Lac$^+$ adaptive mutation. This small effect can be attributed to experimental variability and was not repeated in a separate experiment, in which the same *holE* strain displayed frequencies of adaptive Lac reversion identical to the *holE*$^+$ strain (data not shown). Error bars represent one SEM (not visible if smaller than the data point). **B.** Little change in the numbers of viable frameshift-bearing cells occurred during the experiment. The *holE*$^+$ and *holE* strains correspond to SMR506, SMR1315, respectively (APPENDIX I).

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APPENDIX IV*

ESCHERICHIA COLI SINGLE-STRAND EXONUCLEASES EXOI, EXOVII, AND RECJ ARE NOT ESSENTIAL FOR MISMATCH REPAIR *IN VIVO*

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[*] A version of this appendix has been submitted for publication: Harris, R.S., K.J. Ross, M.-J. Lombardo, and S.M. Rosenberg, submitted to *J. Bacteriol.*

The methyl-directed mismatch repair (MMR) system of *Escherichia coli* is a key enforcer of genetic stability. MMR corrects DNA polymerase mistakes (Modrich, 1991) and prevents recombination of partially diverged DNA sequences (Rayssiguier *et al.*, 1989; Matic *et al.*, 1995). *E. coli* strains lacking any essential component of this system, MutH, MutL, MutS, or MutU (helicase II, UvrD), display a mutator phenotype in which mutation rates are 100- to 1000-fold above normal (Modrich, 1991; Schaaper, 1993). Such strains are also better able to recombine partially-diverged DNA sequences (*e.g.* Rayssiguier *et al.*, 1989; Matic *et al.*, 1995). Both the elevated mutation rate and the relaxed sequence-stringency of recombination of mutator strains may contribute to pathogenesis (LeClerc *et al.*, 1996). Homologues of *E. coli*'s MutS and MutL mismatch repair proteins have been identified in yeast and in human cells and, as predicted from studies in *E. coli*, their absence results in increased mutation, genome instability, and cancer (reviewed by Modrich, 1994).

Biochemical studies have resulted in the following model for the mechanism of methyl-directed mismatch repair in *E. coli* (Grilley *et al.*, 1993; reviewed by Fang *et al.*, 1993; Modrich, 1994; 1995; Linn, 1996; Rupp, 1996): Repair is initiated by binding of MutS to the mismatch, MutL to MutS, and MutH to the closest d(GATC) sequence. An incision is made by MutH 5' to the d(GATC) on an unmethylated DNA strand. The nicked DNA strand is displaced by the coordinated activities of MutS, MutL, and MutU and degraded by exonucleases specific for single-strand DNA. The exonuclease required depends on the position of the incision relative to the mismatch: if located 3', repair requires the 3' to 5' exonucleolytic activity of exonuclease I (ExoI); if located 5', repair requires the 5' to 3' exonucleolytic activity of either RecJ or exonuclease VII (ExoVII). The final steps in mismatch repair require the activities of single-strand DNA binding protein (SSB), DNA polymerase III, and DNA ligase. This model for methyl-directed

MMR in *E. coli* is significant, not only because it allows organization of existing *E. coli* data into a coherent picture, but also because it provides a framework on which studies in other organisms are based. Not all aspects of this model have been verified *in vivo* as yet.

The requirement *in vitro* for ExoI, ExoVII, or RecJ predicts that cells lacking all three exonucleases will display a mutator phenotype similar to *mutH*, *mutL*, *mutS*, or *mutU* mutants. To test this prediction, we created the first precise null allele of *xseA* (*ΔxseA18*::*amp*, TABLE IV-1), the gene encoding the large subunit of ExoVII. *ΔxseA18*::*amp* provides a well-defined alternative to *Δ(xseA-guaB)* (KLC381, Vales *et al.*, 1979) a null allele of *xseA* which complicates comparison with *xseA*$^+$ by having an additional deficiency in DNA (guanine) metabolism. We also used the first precise null allele of *xonA* (*sbcB*) (*ΔxonA300*::*CAT*, Razavy *et al.*, 1996) and a null allele of *recJ* (*recJ284*::Tn*10*, Lovett and Clark, 1984) the genes encoding ExoI and RecJ respectively (TABLE IV-1). The presence of the null alleles in the triple mutants was confirmed by P1 transduction of each mutation into genetic backgrounds in which the following characteristic phenotypes were observed: *recJ284*::Tn*10* makes *recB21 recC22 sbcB15 sbcC201* strains extremely UV sensitive (Lovett and Clark, 1984); *xonA* null mutations decrease transductional recombination *via* the RecF pathway (Benson and Roth, 1994); and *xseA* mutations enhance sensitivity to low concentrations of nalidixic acid (Chase and Richardson, 1977). In two separate *E. coli* K-12 strain backgrounds, we found that cells lacking ExoI, ExoVII, and RecJ displayed mutation rates similar to their *xonA*$^+$ *xseA*$^+$ *recJ*$^+$ parents (TABLE IV-2). In contrast, isogenic strains lacking MutL, an essential component of MMR *in vivo*, showed greatly elevated mutation rates. Thus, the activities of ExoI, ExoVII, and RecJ are not essential for mismatch repair *in vivo*.

These results could indicate, first, that another, as yet, uncharacterized exonuclease(s) is sufficient for MMR (Cooper *et al.*, 1993). The observation that cell extracts prepared from a *xonA* mutant can repair a mismatch located to the 5' side of the incision in the unmethylated DNA strand (Cooper *et al.*, 1993) also supports this idea. However ExoVII was functional in that strain and although this exonuclease does not manifest 3' nuclease activity in the purified mismatch repair system (Cooper *et al.*, 1993), the enzyme possesses both 3' and 5' exonuclease activities (Chase and Richardson, 1974) and might manifest both in crude extracts. Alternatively, it could be that single-strand exonuclease activity is not required for MMR *in vivo*. Displacement of the unmethylated DNA strand by MutU may be unfavorable *in vitro*, perhaps because the displaced single-strand DNA can reanneal. Exonuclease activity might then be required to degrade the displaced DNA strand. This requirement might be bypassed *in vivo* if MutU, MutS, and MutL could remove the unmethylated DNA strand completely (for example, from the original incision to a nick, or a second incision, downstream of the mismatch). SSB could function to prevent the displaced DNA strand from reannealing, thereby clearing the way for DNA resynthesis. Nucleases might still degrade the displaced single-strand, but this would not be an obligate step in MMR. Thus, the coordinated activities of MutH, MutL, MutS, MutU, SSB, a DNA polymerase, and DNA ligase may be sufficient for MMR *in vivo*.

TABLE IV-1. *Escherichia coli* K-12 strains and plasmids.

Strain or plasmid	Relevant genotype or characteristics	Reference or construction
E. coli strains		
AB1157	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	Bachmann, 1996
FC40	*ara Δ(lac-proAB)*$_{\mathrm{XIII}}$ *thi* Rif^r [F' *proAB*$^+$ *lacI33ΩlacZ*]	Cairns and Foster, 1991
JC11450	AB1157, spontaneous Su$^-$	A. J. Clark
SMR91	*mutL211*::Tn*5*	Lab collection
SMR423	*recD1903*::Tn*10*	Lab collection
SMR690	FC40 *recJ284*::Tn*10*	Harris *et al.*, 1994
SMR838	JC11450 *ΔxonA300::CAT*	Lab collection
SMR1403	JC11450 *ΔxonA300::CAT recJ284*::Tn*10*	Razavy *et al.*, 1996
SMR2597	FC40 *Δ(xseA-guaB) zff-3139*::Tn*10kan*	Lab collection
SMR3404	FC40 *mutL211*::Tn*5*	FC40 x P1 (SMR91)
SMR3465	*recD1903*::Tn*10 ΔxseA18::amp*	This study[a]

TABLE IV-1 contd.:

SMR3472	FC40 *ΔxseA18*::*amp*	SMR2597 x P1(SMR3465)[b]
SMR3481	FC40 *ΔxonA300*::*CAT recJ284*::Tn*10 ΔxseA18*::*amp*	FC40 x P1 (SMR838), P1(SMR690), P1(SMR3472)
SMR3488	JC11450 *ΔxonA300*::*CAT recJ284*::Tn*10 ΔxseA18*::*amp*	SMR1403 x P1(SMR3472)
SMR3524	JC11450 *mutL211*::Tn*5*	JC11450 x P1 (SMR3404)
Plasmids		
pMJ3	pACYC184 derivative containing *xseA* and *guaBA*	This study[c]
pMJ6	pMJ3 derivative containing *ΔxseA18*::*amp*	This study[d]

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[a] Constructed by transforming SMR423 with the 5kb *Ava*I-*Bam*HI fragment of pMJ6 which contains *ΔxseA18*::*amp* and selecting ampicillin-resistant transformants (Russell *et al.*, 1989).

[b] This transduction confirmed the chromosomal location of *ΔxseA18*::*amp* as all Amp^r transductants were Gua^+ and the expected linkage to *zff-3139*::Tn*10kan* was observed.

[c] A 5kb *Bgl*I-*Bam*HI fragment from Kohara phage 1427 (λ8E3, Kohara et al., 1987) containing *xseA* and *guaBA* was ligated into *Bgl*I-*Bam*HI-digested pACYC184. *Bgl*I 3' overhangs were removed with T4 DNA polymerase.

[d] The 690bp *Eag*I-*Afl*III fragment of *xseA* [(Chase *et al.*, 1986); GENBANK accession #J02599] was replaced with an *Eag*I-*Afl*III- digested 1035bp PCR fragment containing the *bla* gene of pBR322. *bla* was amplified using primers that create *Eag*I and *Afl*III sites (*Eag*I primer 5'-3'GTACGGCCGAGTAAACTTGGTCTGACA; *Afl*III primer 5'-3'ATGCTTAAGTAGACGTCA-GGTGGCACT).

TABLE IV-2. Mutation rates.

Strain	Genetic background	Relevant genotype	Expt no.	No. of cultures	Mutation rate[a] [(mutations /cell/generation) x 10^{-10}]		
					Nal^r	Str^r	Arg$^+$
SMR122	JC11450	*xonA*$^+$ *xseA*$^+$ *recJ*$^+$ *mutL*$^+$	1	25	1.5	NA	12
			2	25	1.2	NA	11
			3	25	3.7	NA	45
SMR3488	JC11450	Δ*xonA300*::*CAT* *recJ284*::Tn*10* Δ*xseA18*::*amp*	1	25	< 1.6[c]	NA	44
			2	25	< 1.2[c]	NA	36
			3	25	3.0	NA	59
SMR3524	JC11450	*mutL211*::Tn*5*	1	25	580	NA	740
			2	25	700	NA	430
			3	25	700	NA	220

TABLE IV-2 contd.:

SMR506	FC40	*xonA*$^{+}$ *xseA*$^{+}$ *recJ*$^{+}$ *mutL*$^{+}$	3	25	5.0	1.3	NA
			4	25	4.7	0.83	NA
			5	25	3.6	0.82	NA
SMR3481	FC40	Δ*xonA300*::*CAT* *recJ284*::Tn*10* Δ*xseA18*::*amp*	3	25	2.4	0.74	NA
			4	25	$< 2.9^{c}$	$< 1.0^{c}$	NA
			5	25	$< 3.8^{c}$	$< 1.1^{c}$	NA
SMR3404	FC40	*mutL211*::Tn*5*	3	25	1100	91	NA
			4	25	1000	40	NA
			5	25	1200	53	NA

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[a] Nalidixic acid resistant (Nal^{r}) and streptomycin resistant (Str^{r}) colonies were selected on LBH plates (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 2µg/ml thymine, 1.5% agar, pH 7) supplemented with 40µg/ml nalidixic acid or 100µg/ml streptomycin. Arginine prototrophs (Arg^{+}) were selected on minimal M9 plates (Miller, 1992) supplemented with 0.1% glycerol and 5µg/ml of the appropriate amino acids. Mutants were scored after ca. 24 hours (LBH) or 72 hours (M9) of incubation at 37∞C. Mutation rates were calculated using the method of the median as modified by von Borstel (1978).

[b] Not applicable (JC11450 is Str^{r} and FC40 is Arg^{+}).

[c] In these cases more than half of the cultures produced no mutant colonies and mutation rate was calculated using a median of <1 and these rates are thus overestimates and are preceded by "<".

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